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Nitrophenols: Ambient Water Quality Criteria

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NITROPHENOLS

Ambient Water Quality Criteria

Criteria and Standards Division
Office of Water Planning and Standards
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Section 304(a) of the Clean Water Act (33 U.S.C. 1314(a)), requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for nitrophenols. It presents concentration criteria for the protection of fresh water and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A section 304(a) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and scientific judgement. It does not reflect consi....

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CRITERION DOCUMENT

NITROPHENOLS

CRITERIA

Aquatic Life

2-nitrophenol

The data base for freshwater aquatic life is insufficient to allow use of the Guidelines. The following recommendation is inferred from toxicity data on 4-nitrophenol and saltwater organisms.

For 2-nitrophenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 2,700 µg/l as a 24-hour average and the concentration should not exceed 6,200 µg/l at any time.

For saltwater aquatic life, no criterion for 2-nitrophenol can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

4-nitrophenol

The data base for freshwater aquatic life is insufficient to allow use of the Guidelines. The following recommendation is inferred from toxicity data on 4-nitrophenol and saltwater organisms.

For 4-nitrophenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 240 µg/l as a 24-hour average and the concentration should not exceed 550 µg/l at any time.

For 4-nitrophenol the criterion to protect saltwater aquatic life as derived using the Guidelines is 53 µg/l as a 24-hour average and the concentration should not exceed 120 µg/l at any time.

2,4-dinitrophenol

The data base for freshwater aquatic life is insufficient to allow use of the Guidelines. The following recommendation is inferred from toxicity data on 4-nitrophenol and saltwater organisms.

For 2,4-dinitrophenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 79 µg/l as a 24-hour average and the concentration should not exceed 180 µg/l at any time.

The data base for saltwater aquatic life is insufficient to allow use of the Guidelines. The following recommendation is inferred from toxicity data on 4-nitrophenol and saltwater organisms.

For 2,4-dinitrophenol the criterion to protect saltwater aquatic life as derived using procedures other than the Guidelines is 37 µg/l as a 24-hour average and the concentration should not exceed 84 µg/l at any time.

2,4-dinitro-6-methylphenol

The data base for freshwater aquatic life is insufficient to allow use of the Guidelines. The following recommendation is inferred from toxicity data on 4-nitrophenol and saltwater organisms.

For 2,4-dinitro-6-methylphenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 57 µg/l as a 24-hour average and the concentration should not exceed 130 µg/l at any time.

For saltwater aquatic life, no criterion for 2,4-dinitro-6-methylphenol can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

2,4,6-trinitrophenol

The data base for freshwater aquatic life is insufficient to allow use of the Guidelines. The following recommendation is inferred from toxicity data on 4-nitrophenol and saltwater organisms.

For 2,4,6-trinitrophenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 1,500 μg/l as a 24-hour average and the concentration should not exceed 3,400 μg/l at any time.

The data base for saltwater aquatic life is insufficient to allow use of the Guidelines. The following recommendation is inferred from toxicity data on 4-nitrophenol and saltwater organisms.

For 2,4,6-trinitrophenol the criterion to protect saltwater aquatic life as derived using procedures other than the Guidelines is 150 μg/l as a 24-hour average and the concentration should not exceed 340 μg/l at any time.

Human Health

To protect human health from the adverse effects of various nitrophenols ingested in contaminated water and fish, suggested criteria are as follows:

Mononitrophenols	no criterion
Dinitrophenols	68.6 μg/l
Trinitrophenols	10 μg/l
Dinitrocresols	12.8 μg/l

MONONITROPHENOLS

Introduction

Mononitrophenol has three isomeric forms, distinguished by the position of the nitro group on the phenolic ring. Three isomeric forms are possible, namely 2-nitrophenol, 3-nitrophenol, and 4-nitrophenol. The compounds are also commonly referred to as o-nitrophenol, m-nitrophenol, and p-nitrophenol, respectively.

Commercial synthesis of 2-nitrophenol and 4-nitrophenol is accomplished through the hydrolysis of the appropriate chloronitrobenzene isomers with aqueous sodium hydroxide at elevated temperatures (Howard, et al. 1976). Production of 3-nitrophenol is achieved through the diazotization and hydrolysis of m-nitroaniline (Matsuguma, 1967). The mononitrophenol isomers are used in the United States primarily as intermediates for the production of dyes, pigments, pharmaceuticals, rubber chemicals, lumber preservatives, photographic chemicals, and pesticidal and fungicidal agents (U.S. Int. Trade Comm., 1976). As a result of this use pattern, the major source for environmental release of mononitrophenols is likely from production plants and chemical firms where the compounds are used as intermediates. The mononitrophenols may also be inadvertently produced via microbial or photodegradation of pesticides which contain mononitrophenol moieties. Approximately 10 to 15 million pounds of 2-nitrophenol are produced annually (Howard, et al. 1976) for uses including synthesis of o-aminophenol, o-nitroanisole, and other dye stuffs (Matsuguma, 1967; Howard, et al. 1976). Although production figures for 3-nitrophenol are not available, Hoecker, et al. (1977) estimate that production is less than one million pounds annually. 3-Nitrophenol is used in the manufacture of dye intermediates such as anisidine and m-aminophenol (Kouris and Northcott, 1963; Matsuguma, 1967). 4-Nitrophenol is probably the most important of the mononitrophenols in terms of quantities used and potential environmental contamination. Demand for 4-nitrophenol was 35,000,000 pounds in 1976 and production is projected to increase to 41,000,000 pounds by 1980 (Chem. Market. Reporter, 1976). Most of the 4-nitrophenol produced (87 percent) is used in the manufacture of ethyl and methyl parathions. Other uses (13 percent) include the manufacture of dye-stuffs and n-acetyl-p-aminophenol (APAP) and leather treatments. A possible source of human exposure to 4-nitrophenol is as a result of microbial or photodegradation of the parathions. In vivo production of 4-nitrophenol following absorption of parathion or other pesticides by humans is another possible source of human exposure.

Physical and chemical properties of the mononitrophenols are summarized in Table 1.

TABLE 1

Properties of Mononitrophenols

	2-Nitrophenol	3-Nitrophenol	4-Nitrophenol
Formula	$C_6H_5NO_3$	$C_6H_5NO_3$	$C_6H_5NO_3$
Molecular Weight	139.11	139.11	139.11
Melting Point (°C)	44-45	97	113-114
Boiling Point	214-216	194	279
Density	1.485	1.485	1.479
Water Solubility (g/l)	0x3.2 at 38°C 1x0.8 at 100°C	1x3.5 at 25°C 13x3.0 at 90°C	0x8.04 at 15°C 1x6.0 at 25°C
Vapor Pressure	1 mm Hg at 49.3°C		
Ka	7.5×10^{-8}	5.3×10^{-9}	7×10^{-8}

DINITROPHENOLS

Six isomeric forms of dinitrophenol are possible, distinguished by the position of the nitro groups on the phenolic ring. Of the six possible dinitrophenol isomers, 2, 4-dinitrophenol is by far the most important. The most recent production figure for 2,4-dinitrophenol is 863,000 lb reported by the U.S. International Trade Commission (1968). Approximate consumption per year is estimated at 1,000,000 lb (Howard, et al, 1976). 2, 4-dinitrophenol is used primarily as a chemical intermediate for the production of sulfur dyes, azo dyes, photochemicals, pest control agents, wood preservatives, and explosives (Matsuguma, 1967; Perkins, 1919; Springer, et al. 1977a,b).

Production figures and usage data for the remaining five dinitrophenol isomers are not available. It is reasonable to assume that production and usage of these compounds are extremely limited in the United States.

Commercial synthesis of 2,4-dinitrophenol is accomplished by the hydrolysis of 2,4-dinitro-1-chlorobenzene with sodium hydroxide at 95 to 100°C (Matsuguma, 1967). As a result of the use pattern of 2,4-dinitrophenol (2,4-DNP) the major source for environmental release of 2,4-DNP is likely from production plants and chemical firms where the compound is used as an intermediate. It is possible that 2,4-DNP may also be produced via microbial or photodegradation of compounds which contain the dinitrophenol moiety, such as Parathion (Gomaa and Faust, 1972). 2,4-DNP has also been identified as an impurity in technical preparations of the herbicide DNPP (2-isopropyl-4,6-dinitrophenol) by Mosinska and Kotarski (1972).

The physical and chemical properties of the dinitrophenol isomers are summarized in Table 2.

TABLE 2

Properties of Dinitrophenol Isomers[a]

Isomer	m.p. (°C)	K (at 25°C)	Water Solubility (g/l)	Density
2,3-Dinitrophenol	144	1.3×10^{-5}	2.2	1.681
2,4-Dinitrophenol	114-115 (sublimes)	1.0×10^{-4}	0.79	1.683
2,5-Dinitrophenol	104	7×10^{-6}	0.68	
2,6-Dinitrophenol	63.5	2.7×10^{-4}	0.42	
3,4-Dinitrophenol	134	4.3×10^{-5}	2.3	1.672
3,5-Dinitrophenol	122-123	2.1×10^{-4}	1.6	1.702

[a] Source: Harvey, 1959; Windholz, 1976; Weast, 1975.

TRINITROPHENOLS

Six isomeric forms of trinitrophenol are possible, distinguished by the position of the nitro groups relative to the hydroxy group on the six carbon benzene ring. The five isomers are: 2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6- and 3,4,5-trinitrophenol. Production volumes for the trinitrophenols are not available. Usage of the trinitrophenol isomers is apparently limited to 2,4,6-trinitrophenol, otherwise known as picric acid. In fact, a comprehensive search of the literature failed to detect a single citation dealing with any of the trinitrophenol isomers except picric acid. Consequently, the only information on these isomers presented in this document is the chemical and physical properties found in Table 3.

According to Matsuguma (1967) picric acid has found usage as: a dye intermediate, explosive, analytical reagent, germicide, fungicide, staining agent and tissue fixative, tanning agent, photochemical, pharmaceutical, and a process material for the oxidation and etching of iron, steel and copper surfaces. The extent to which picric acid finds usage in any of these applications at the present time is unknown.

TABLE 3

Properties of Trinitrophenols

2,3,4-Trinitrophenol	
Molecular Weight	229.11
2,3,5-Trinitrophenol	
Molecular Weight	229.11
Melting Point	119-120°C
2,3,6-Trinitrophenol	
Molecular Weight	229.11
Melting Point	119°C
Water Solubility	
Room Temperature	Slightly Soluble
Hot Water	Very Soluble
2,4,5-Trinitrophenol	
Molecular Weight	229.11
Melting Point	96°C
Water Solubility	
Room Temperature	Slightly Soluble
Hot Water	Soluble
2,4,6-Trinitrophenol	
Molecular Weight	229.11
Melting Point	122-123°C
Boiling Point	Sublimates: Explodes at 300°C
Vapor Pressure	1 mm Hg at 195°C
Density	1.763 g/cm^3
Water Solubility	
Room Temperature	1.28 g/l
100°C	6.7 g/l

DINITROCRESOLS

Dinitro-ortho-cresol is a yellow crystalline solid derived from o-cresol. There are six possible isomers but the 4,6-dinitro-o-cresol isomer is the only one of any commercial importance. In fact, a comprehensive search of the literature failed to reveal information on any of the other five dinitro cresol isomers.

4,6-dinitro-o-cresol (hereafter referred to as DNOC) is produced either by sulfonation of o-cresol followed by treatment with nitric acid or by treatment of o-cresol in glacial acetic acid with nitric acid at low temperature. Some important chemical and physical properties of DNOC are shown in Table 4.

TABLE 4

Properties of 4,6-Dinitro-o-cresol

Molecular Weight	198.13
Appearance	Yellow Solid
Melting Point	85.8°C
Vapor Pressure	0.000052 mm Hg at 20°C
Water Solubility	100 mg/l at 20°C
pKa	4.46

An excellent review of the toxicological effects of DNOC on human and laboratory animals has recently been published by the National Institute for Occupational Safety and Health (1978). In view of the comprehensive coverage of both English and foreign language literature, no attempt will be made to duplicate this impressive effort within this criterion document. Key papers used for criterion formulation will be cited, where appropriate, and frequent reference to the NIOSH review will be used where the available literature does not contain information directly relevant to criteria formulation.

DNOC usage in the U.S. has declined in recent years because the compound is highly toxic to plants in the growing stage and nonselectively kills both desirable and undesirable vegetation. Additionally, the compound is highly toxic to humans and is considered one of the more dangerous agricultural pesticides.

The Environmental Protection Agency has no record of DNOC being currently manufactured in the United States for use as an agricultural chemical. Imports of DNOC have also decreased in recent years; from 217,899 lbs. in 1972 to 146,621 lbs. in 1973 and then to 30,442 lbs. in 1976 (Natl. Inst. Occup. Safety Health, 1978). Since DNOC is not manufactured in the U.S., pesticide formulators and sprayers are the major groups with potential occupational exposure to DNOC.

DNOC is used primarily as a blossom-thinning agent on fruit trees and as a fungicide, insecticide, and miticide on fruit trees during the dormant season. NIOSH (1978) estimates that 3,000 workers in the U.S. are potentially exposed to DNOC. In view of the small amount of DNOC used in the U.S., exposure of the general public is expected to be minimal.

Few data are available regarding the breakdown of nitrophenols by natural communities of microorganisms. A number of researchers have isolated microorganisms capable of using nitrophenols as a sole source of carbon in pure culture (Simpson and Evans, 1953; Raymond and Alexander, 1971; Chambers, et al. 1963; Guillaume, et al. 1963). However, the significance of such studies as related to the stability of nitrophenols in the environment is not known.

Several investigators have shown that individual species of aerobic and anaerobic bacteria, including *Azotabacter chroococcum* and *Clostridium butyrium*, and the fungus *Fusarium*, are capable of reducing 2,4-dinitrophenol in culture (Radler, 1955; Lehmber, 1956; Madhosingh, 1961). However, the precise pathway for metabolic degradation is not known. Jensen and Lautrup-Larson (1967) found that *Arthrobacter simplex*, *Pseudomonas*, and *Arthrobacter* were able to metabolize 2,4-dinitrophenol and 2,4,6-trinitrophenol, forming nitrite.

The actual degradation pathway of dinitro-o-cresol has been investigated by Tewfik and Evans (1966) in pure cultures of microorganisms. It was reported that in *Pseudomonas* sp. degradation proceeded by way of formation of an aminocresol. In *Arthrobacter simplex*, a hydroxylated catechol is formed prior to ring cleavage.

The significance of such studies as related to the stability of nitrophenols in the environment is not known. Certain investigators have postulated that ambient nitrophenol concentrations may be too low to induce the appropriate microbial enzymes necessary to facilitate population growth and metabolism of the compounds (U.S. EPA).

Information regarding the mobility and persistence of nitrophenols in natural soil and water environments is limited. Based upon experimentally determined solubilities and sorption characteristics, the persistence of some of the nitrophenols might be estimated. For example, although 2-nitrophenol is soluble in water, it has also been shown to be strongly attracted through hydrogen bonding to montmorillonite clays, perhaps reducing its movement through the groundwater regime (Saltzman and Yariv, 1975; Yariv, et al. 1966). However, these estimates do not consider the data abailable on microbial, photolytic, and oxidative degradation available in the literature.

No measured steady-state data are available regarding the bioconcentration of nitrophenols. However, BCF's are estimated in this document using the octanol-water partition coefficients. Only limited no data are available on the levels of nitrophenols in municipal effluents or treated drinking waters.

None of the nitrophenols addressed in this document is found to be carcinogenic, mutagenic, or teratogenic; however, because of their widespread use as agricultural chemicals, their toxicity to microorganisms, fish, and mammals, the nitrophenols pose a potential threat to aquatic and terrestrial life, including man.

REFERENCES

Chambers, C.W., et al. 1963. Degradation of aromatic compounds by phenol-adapted bacteria. Jour. Water Pollut. Control Fed. 35: 1517.

Gomaa, H.M., and S.D. Faust. 1972. Chemical hydrolysis and oxidation of parathion and paraoxon in aquatic environments. Adv. Chem. Ser. Vol. III. Iss. Fate Org. Pestic. in the Aquatic Environ.

Guillaume, J., et al. 1963. Oxidation of p-nitrophenol by certain Mycobacteria. Compt. Rend. 256: 1634.

Harvey, D.G. 1959. On the metabolism of some aromatic nitro compounds by different species of animal. Part III. The toxicity of the dinitrophenols, with a note on the effects of high environmental temperatures. Jour. Pharm. Pharmacol. 11: 462.

Hoecker, J.E., et al. 1977. Information profiles on potential occupational hazards. Nat. Inst. Occup. Safety Health, Cincinnati, Ohio.

Howard, H., et al. 1976. Investigation of selected potential environmental contamination: Nitroaromatics. Off. Tox. Subs. U.S. Environ. Prot. Agency, Washington, D.C.

Jensen, H.L., and G. Lautrup-Larson. 1967. Microorganisms that decompose nitro-aromatic compounds, with special reference to dinitro-o-cresol. Acta Agric. Scand. 17: 115.

Kouris, C.S., and J. Northcott. 1963. Aniline and its derivatives. Page 411 in Kirk-Othmer Encyclopedia of Chemical Technology. 2nd ed. Vol 2.

Lehmber, C. 1956. Untersuchungen uber die Winbung von Ascorbunsaure, Stoffwechselgifren and Anderen Faktoren auf den Staffwechsel von Clostridium butyrium. Beif. Arch. Mikrobiol 24: 323.

Madhosingh, C. 1961. The metabolic detoxification of 2,4-dinitrophenol by Fusarium oxysporum. Can. Jour. Microbiol. 7: 553.

Matsuguma, H.J. 1967. Nitrophenols. Page 888 in Kirk-Othmer Encyclopedia of Chemical Technology. 2nd. ed. Vol. 13.

Mosinska, K., and A. Kotarski. 1972. Determination of 2-isopropyl-4,6-dinitrophenol and 2,4-DNP in herbicides and in technical 2-isopropyl-4,6-DNP by TCL. Chemia Analityezma 17: 327.

National Institute for Occupational Safety and Health. 1978. Criteria for a recommended standard: Occupational exposure to dinitro-ortho-cresol. Dep. Health Edu. Welfare, Washington, D.C.

Perkins, R.G. 1919. A study of the munitions intoxications in France. Pub. Health Rep. 34: 2335.

Radler, F. 1955. Untersuchunger uber den verlauf der stoffwech Selvorgangebei Azotobacter chroococcum. Beig. Arch. Microbiol. 22: 335.

Raymond, D.G.M., and M. Alexander. 1971. Microbial metabolism and cometabolism of nitrophenols. Pestic. Biochem. Physiol. 1: 123.

Saltzman, S., and S. Yariv. 1975. Infrared study of the sorbtion of phenol and p-nitrophenol by montmorillonite. Soil Sci. Soc. Am. Proc. 39: 474.

Simpson, J.R., and W.C. Evans. 1953. The metabolism of nitrophenols by certain bacteria. Biochem. Jour. 55: 24.

Springer, E.L., et al. 1977a. Chemical treatment of chips for outdoor storage. Evaluation of sodium n-methyldithiocarbomate and sodium 2,4-dinitrophenol treatment. Tapi 60: 88.

Springer, E.L. et al. 1977b. Evaluation of chemical treatments to prevent deterioration of wood chips during storage. Tapi 60: 93.

Tewfik, M.S., and W.C. Evans. 1966. The metabolism of 3,5-dinitro-o-cresol (DNOC) by soil microorganisms. Biochem. Jour. 99: 31.

U.S. EPA. 1976. Investigation of selected potential environmental contaminants: nitroaromatics. Final Rep. Off. Tox. Subst. Washington, D.C.

U.S. International Trade Commission. 1967-73. Synthetic organic chemicals: U.S. production and sales. Washington, D.C.

U.S. International Trade Commission. 1976. Imports of benzenoid chemicals and products, 1974. Publ. No. 762. Washington, D.C.

Weast, R.C., ed. 1975. Handbook of chemistry and physics. 57th ed. CRC Press.

Windholz, M., ed. 1976. The Merck Index. 9th ed. Merck and Co., Rahway, N.J.

Yariv, S., et al. 1966. Infrared study of the absorption of benzoic acid and nitrobenzene in montomorillonite. Israel Jour. Chem. 4: 201.

AQUATIC LIFE TOXICOLOGY*

FRESHWATER ORGANISMS

Introduction

Although fish and invertebrate acute toxicity data and plant toxicity data are available for the groups of organic compounds which contain various numbers of nitro groups substituted into the aromatic ring of a phenol or cresol, collectively referred to as nitrophenols, there are only limited data available for each individual nitrophenol. There are no data available dealing with chronic effects of any nitrophenol on freshwater aquatic organisms, and no suitable substitute chronic value can be determined from available toxicity information. The derivation of a single criterion which would protect freshwater aquatic organisms from all nitrophenols is impractical because of the limited toxicity data for each compound and because of the wide differences in toxicity results obtained for individual nitrophenols.

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*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life [43 FR 21506 (May 18, 1978) and 43 FR 29028 (July 5, 1978)] in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are the calculations for deriving various measures of toxicity as described in the Guidelines.

Acute Toxicity

The fish acute toxicity data base (Table 1) consists of eight LC50 values for five nitrophenols and two species of freshwater fish. Using adjusted values for the three nitrophenols for which data are available for both fish species, bluegills were found to be at least 13 times more sensitive than were fathead minnows although the tests with fathead minnows were flow-through with measured concentrations and the tests with bluegills were static without measured concentrations. Comparisons of adjusted LC50 values (Table 1) indicate that 2,4-dinitro-6-methylphenol is the most toxic nitrophenol with 96-hour LC50 values of 126 µg/l (U.S. EPA, 1978) and 2,040 µg/l (Phipps, et al. manuscript) for bluegills and fathead minnows, respectively. 2,4-dinitro-6-methylphenol is followed in order of decreasing toxicity by 2,4-dinitrophenol, 4-nitrophenol, 2-nitrophenol, and 2,4,6-trinitrophenol. The adjusted 96-hour LC50 values for 2,4-dinitrophenol are 339 µg/l for bluegills (U.S. EPA, 1978) and 16,720 µg/l for fathead minnows (Phipps, et al. manuscript). Toxicity differences between the various mononitrophenol compounds are indicated by the adjusted bluegill LC50 values of 4,527 µg/l for 4-nitrophenol (U.S. EPA, 1978) and 24,139 µg/l for 2-nitrophenol (Lammering and Burbank, 1960). The high 2,4,6-trinitrophenol adjusted LC50 value of 91,299 µg/l for bluegills (U.S. EPA, 1978) indicates the toxicity of nitrophenols does not increase directly with increasing nitro-group substitution. The Final Fish Acute Values for 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2,4-dinitro-6-methylphenol, and 2,4,6-trinitrophenol are 6,200, 4,200, 610, 130, and 23,000 µg/l, respectively.

The data base for invertebrate species (Table 2) contains seven data points for four nitrophenol compounds with two invertebrate species. The order of toxicity of these four nitrophenols is the same for invertebrate species as observed with fish. An unspecified dinitromethylphenol is the most toxic and is followed in order of decreasing toxicity by 2,4-dinitrophenol, 4-nitrophenol, and 2,4,6-trinitrophenol. For 2,4-dinitrophenol, the two adjusted LC50 values for daphnids are quite close and are 3,989 µg/l (Kopperman, et al. 1974) and 3,464 µg/l (U.S. EPA, 1978). The toxicity of 4-nitrophenol to daphnids showed greater variation and adjusted LC50 values are 7,111 µg/l (Kopperman, et al. 1974) and 18,549 µg/l (U.S. EPA, 1978). As previously noted with fish, 2,4,6-trinitrophenol, with an LC50 value of 71,741 µg/l for daphnids (U.S. EPA, 1978), is much less toxic than other nitrophenols. The Final Invertebrate Acute Values for 4-nitrophenol, 2,4-dinitrophenol, 2,4-dinitro-6-methylphenol, and 2,4,6-trinitrophenol are 550, 180, 130, and 3,400 µg/l, respectively.

The data indicate that the Final Invertebrate Acute Values for nitrophenols are lower than or equivalent to, as in the case of 2,4-dinitro-6-methylphenol, comparable values for fish. Thus, when a Final Invertebrate Acute Value exists, it becomes the Final Acute Value. The Final Fish Acute Value of 6,200 µg/l for 2-nitrophenol is the Final Acute Value for that compound.

Chronic Toxicity

There are no data available on the chronic effects of any of the various nitrophenols on freshwater aquatic life.

Plant Effects

Plant toxicity values (Table 3) are lower, in certain instances, than adjusted acute toxicity values for fish and invertebrate species. However, no plant toxicity effects are lower than the Final Fish or Final Invertebrate Acute Values. Tests which elicited the relative toxicity of the three isomeric forms of mononitrophenols to plants (Huang and Gloyna, 1967) indicated that chlorophyll synthesis in the alga, *Chlorella pyrenoidosa*, was inhibited at 25,000 µg/l by 4-nitrophenol, at 35,000 µg/l by 2-nitrophenol, and at 50,000 µg/l by 3-nitrophenol. Studies with three species of algae (Table 3) indicate that 4-nitrophenol is more toxic to plants than is 2,4-dinitrophenol. The one exception to this toxicity trend was determined by Simon and Blackman (1953), who found that 50 percent growth reduction in duckweed, *Lemna minor*, occurred at 2,4-dinitrophenol and 4-nitrophenol concentrations of 1,472 µg/l and 9,452 µg/l, respectively. As observed with fish and invertebrate species, 2,4,6-trinitrophenol was less toxic to the alga, *Selenastrum capricornutum* than was either 4-nitrophenol or 2,4-dinitrophenol (U.S. EPA, 1978).

Residues

No measured steady-state bioconcentration factors (BCFs) are available for any nitrophenol. BCFs can be estimated using the octanol-water partition coefficients of 32, 150 and 110 for 2,4-dinitrophenol, 2,4-dinitro-6-methylphenol, and 2,4,6-trinitrophenol, respectively. These coefficients are used to derive estimated BCFs of 8.1, 26, and 21 for 2,4-dinitrophenol, 2,4-dinitro-6-methylphenol and 2,4,6-trinitrophenol, respectively, for aquatic organisms that contain about 8 percent lipids. If it is known that the diet of the wildlife of concern contains a significantly different lipid content, appropriate adjustments in the estimated BCFs should be made. No estimates can be made for 2-nitrophenol and 4-nitrophenol.

Miscellaneous

Table 4 contains no data that would be a suitable substitute for a Final Chronic Value for any nitrophenol compound. All data are for short duration (less than 96 hours) and none of the toxicity values are below the lowest adjusted acute toxicity values for fish or invertebrate species. One set of data (Table 4) indicates the relative toxicity of the three isomeric forms of mononitrophenol to fish. Gersdorff (1939) found that 8,000 μg/l of 4-nitrophenol, 24,000 μg/l of 3-nitrophenol, and 33,300 μg/l of 2-nitrophenol produced 42 percent, 53 percent, and 38 percent mortality, respectively, in goldfish after 8 hours.

CRITERION FORMULATION

Freshwater-Aquatic Life

Summary of Available Data

The concentrations below have been rounded to two significant figures.

2-nitrophenol

Final Fish Acute Value = 6,200 µg/l

Final Invertebrate Acute Value = not available

Final Acute Value = 6,200 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 35,000 µg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 35,000 µg/l

0.44 x Final Acute Value = 2,700 µg/l

4-nitrophenol

Final Fish Acute Value = 4,200 µg/l

Final Invertebrate Acute Value = 550 µg/l

Final Acute Value = 550 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 4,900 µg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 4,900 µg/l

0.44 x Final Acute Value = 240 µg/l

2,4-dinitrophenol

Final Fish Acute Value = 610 µg/l

Final Invertebrate Acute Value = 180 µg/l

Final Acute Value = 180 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 1,500 μg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 1,500 μg/l

0.44 x Final Acute Value = 79 μg/l

2,4-dinitro-6-methylphenol

Final Fish Acute Value = 130 μg/l

Final Invertebrate Acute Value = 130 μg/l

Final Acute Value = 130 μg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 50,000 μg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 50,000 μg/l

0.44 x Final Acute Value = 57 μg/l

2,4,6-trinitrophenol

Final Fish Acute Value = 23,000 μg/l

Final Invertebrate Acute Value = 3,400 μg/l

Final Acute Value = 3,400 μg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 62,000 μg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 62,000 μg/l

0.44 x Final Acute Value = 1,500 μg/l

No freshwater criterion can be derived for any nitrophenol using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available.

Results obtained with 4-nitrophenol and saltwater organisms indicate how criteria may be estimated for nitrophenols and freshwater organisms.

For 4-nitrophenol and saltwater organisms 0.44 times the Final Acute Value is less than the Final Chronic Value which is derived from results of an embryo-larval test with the sheepshead minnow. Therefore, it seems reasonable to estimate criteria for nitrophenols and freshwater organisms using 0.44 times the Final Acute Value.

2-nitrophenol

The maximum concentration of 2-nitrophenol is the Final Acute Value of 6,200 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on freshwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2-nitrophenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 2,700 µg/l as a 24-hour average and the concentration should not exceed 6,200 µg/l at any time.

4-nitrophenol

The maximum concentration of 4-nitrophenol is the Final Acute Value of 550 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on freshwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 4-nitrophenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 240 µg/l as a 24-hour average and the concentration should not exceed 550 µg/l at any time.

2,4-dinitrophenol

The maximum concentration of 2,4-dinitrophenol is the Final Acute Value of 180 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on freshwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2,4-dinitrophenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 79 µg/l as a 24-hour average and the concentration should not exceed 180 µg/l at any time.

2,4-dinitro-6-methylphenol

The maximum concentration of 2,4-dinitro-6-methylphenol is the Final Acute Value of 130 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on freshwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2,4-dinitro-6-methylphenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 57 µg/l as a 24-hour average and the concentration should not exceed 130 µg/l at any time.

2,4,6-trinitrophenol

The maximum concentration of 2,4,6-trinitrophenol is the Final Acute Value of 3,400 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on freshwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2,4,6-trinitrophenol the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 1,500 µg/l as a 24-hour average and the concentration should not exceed 3,400 µg/l at any time.

Table 1. Freshwater fish acute values for nitrophenols

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)	Reference
Fathead minnow (juvenile), Pimephales promelas	FT	M	4-Nitrophenol	96	60,510	60,510	Phipps, et al. Manuscript
Fathead minnow (juvenile), Pimephales promelas	FT	M	2,4-Dinitrophenol	96	16,720	16,720	Phipps, et al. Manuscript
Fathead minnow (juvenile), Pimephales promelas	FT	M	2,4-Dinitro-6-methylphenol***	96	2,040	2,040	Phipps, et al. Manuscript
Bluegill (juvenile), Lepomis macrochirus	R	U	2-Nitrophenol	24	66,900	24,139	Lammering & Burbank, 1960
Bluegill, Lepomis macrochirus	S	U	4-Nitrophenol	96	8,280	4,527	U.S. EPA, 1978
Bluegill, Lepomis macrochirus	S	U	2,4-Dinitrophenol	96	620	339	U.S. EPA, 1978
Bluegill, Lepomis macrochirus	S	U	2,4,6-Trinitrophenol	96	167,000	91,299	U.S. EPA, 1978
Bluegill, Lepomis macrochirus	S	U	2,4-Dinitro-6-methylphenol	96	230	126	U.S. EPA, 1978

* S = static, R = renewal, FT = flow-through ***Authors reported results as 4,6-dinitro-o-cresol.

** U = unmeasured, M = measured

Geometric mean of adjusted values:

2-nitrophenol = 24,139 μg/l $\frac{24,139}{3.9}$ = 6,200 μg/l

4-nitrophenol = 16,551 μg/l $\frac{16,551}{3.9}$ = 4,200 μg/l

2,4-dinitrophenol = 2,381 μg/l $\frac{2,381}{3.9}$ = 610 μg/l

2,4,6-trinitrophenol = 91,299 μg/l $\frac{91,299}{3.9}$ = 23,000 μg/l

2,4-dinitro-6-methylphenol = 507 μg/l $\frac{507}{3.9}$ = 130 μg/l

Lowest values from flow-through tests with measured concentrations:

4-nitrophenol = 60,510 μg/l
2,4-dinitrophenol = 16,720 μg/l
2,4-dinitro-6-methylphenol = 2,040 μg/l

Table 2. Freshwater invertebrate acute values for nitrophenols

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (µg/l)	Adjusted LC50 (µg/l)	Reference
Cladoceran, *Daphnia magna*	S	U	4-Nitrophenol	48	8,396	7,111	Kopperman, et al. 1974
Cladoceran, *Daphnia magna*	S	U	4-Nitrophenol	48	21,900	18,549	U.S. EPA, 1978
Cladoceran, *Daphnia magna*	S	U	2,4-Dinitrophenol	48	4,710	3,989	Kopperman, et al. 1974
Cladoceran, *Daphnia magna*	S	U	2,4-Dinitrophenol	48	4,090	3,464	U.S. EPA, 1978
Cladoceran, *Daphnia magna*	S	U	2,4,6-Trinitrophenol	48	84,700	71,741	U.S. EPA, 1978
Cladoceran, *Daphnia magna*	S	U	2,4-Dinitro-6-methylphenol	48	3,120	2,643	U.S. EPA, 1978
Stonefly (naiad), *Pteranarcys californica*	S	U	Dinitromethylphenol***	96	320	271	Sanders & Cope, 1968

* S = static

** U = unmeasured

***This LC50 value was not used in calculating any geometric mean because the dinitromethylphenol tested was not specified. Authors reported results as dinitrocresol.

Geometric mean of adjusted values: 4-nitrophenol = 11,485 µg/l $\frac{11,485}{21} = 550$ µg/l

2,4-dinitrophenol = 3,717 µg/l $\frac{3,717}{21} = 180$ µg/l

2,4,6-trinitrophenol = 71,741 µg/l $\frac{71,741}{21} = 3,400$ µg/l

2,4-dinitro-6-methylphenol = 2,643 µg/l $\frac{2,643}{21} = 130$ µg/l

Table 3. Freshwater plant effects for nitrophenols

Organism	Effect	Concentration (ug/l)	Reference
Alga, *Chlorella pyrenoidosa*	Inhibition of chlorophyll synthesis after 3 days	35,000 2-Nitrophenol	Huang & Gloyna, 1967
Alga, *Chlorella pyrenoidosa*	Inhibition of chlorophyll synthesis after 3 days	50,000 3-Nitrophenol	Huang & Gloyna, 1967
Alga, *Chlorella pyrenoidosa*	Inhibition of chlorophyll synthesis after 3 days	25,000 4-Nitrophenol	Huang & Gloyna, 1967
Alga, *Chlorella pyrenoidosa*	Inhibition of chlorophyll synthesis after 3 days	50,000 2,4-Dinitrophenol	Huang & Gloyna, 1967
Alga, *Chlorella pyrenaidosa*	Inhibition of chlorophyll synthesis after 3 days	50,000 2,4-dinitro-6-methylphenol*	Huang & Gloyna, 1967
Alga, *Chlorella vulgaris*	50% growth inhibition in 80 hrs	6,950 4-Nitrophenol	Dedonder & Van Sumere, 1971
Alga, *Chlorella vulgaris*	70% growth inhibition in 80 hrs	9,200 2,4-Dinitrophenol	Dedonder & Van Sumere, 1971
Alga, *Selenastrum capricornutum*	50% reduction in chlorophyll *a* in 96 hrs	4,190 4-Nitrophenol	U.S. EPA, 1978
Alga, *Selenastrum capricornutum*	50% reduction in chlorophyll *a* in 96 hrs	9,200 2,4-Dinitrophenol	U.S. EPA, 1978
Alga, *Selenastrum capricornutum*	50% reduction in chlorophyll *a* in 96 hrs	41,700 2,4,6-Trinitrophenol	U.S. EPA, 1978

Table 3. (Continued)

Organism	Effect	Concentration (ug/l)	Reference
Duckweed, *Lemna minor*	50% growth reduction	62,550 2-Nitrophenol	Simon & Blackman, 1953
Duckweed, *Lemna minor*	50% growth reduction	9,452 4-Nitrophenol	Simon & Blackman, 1953
Duckweed, *Lemna minor*	50% growth reduction	1,472 2,4-Dinitrophenol	Simon & Blackman, 1953

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*Authors reported results as 4,6-dinitro-o-cresol.

Table 4. Other freshwater data for nitrophenols

Organism	Test Duration	Effect	Result (µg/l)	Reference
Alga, *Chlamydomonas*	30 sec	50% inhibition of flagellar motility	18,400 Dinitrophenol	Marcus & Mayer, 1963
Amoeba, *Amoeba proteus*	24 hrs	46% reduction in ameba containing golgi bodies	92,000 Dinitrophenol	Flickinger, 1972
Amoeba, *Amoeba proteus*	48 hrs	18% mortality	92,000 Dinitrophenol	Flickinger, 1972
Southern bullfrog (tadpole), *Rana grylio*	7 hrs	Increased respiration	5,520 2,4-Dinitrophenol	Lewis & Frieden, 1959
Goldfish, *Carassius auratus*	8 hrs	38% mortality	33,300 2-Nitrophenol	Gersdorff, 1939
Goldfish, *Carassius auratus*	8 hrs	53% mortality	24,000 3-Nitrophenol	Gersdorff, 1939
Goldfish, *Carassius auratus*	8 hrs	42% mortality	8,000 4-Nitrophenol	Gersdorff, 1939

SALTWATER ORGANISMS

Introduction

The three nitrophenols having saltwater data are 4-nitrophenol, 2,4-dinitrophenol and 2,4,6-trinitrophenol. Since 2,4-dinitrophenol is known to uncouple oxidative-phosphosylation, it is not surprising that it is the most toxic compound for both invertebrate and fish species. No invertebrate chronic information could be found and only one study of fish chronic toxicity (4-nitrophenol) is available.

Acute Toxicity

The sheepshead minnow has been exposed for 96 hours (U.S. EPA, 1978) to 4-nitrophenol, 2,4-dinitrophenol, and 2,4,6-trinitrophenol; the adjusted LC50 values are 14,816, 16,073, and 73,258 µg/l, respectively (Table 5). As with freshwater fish (Table 1), 2,4,6-trinitrophenol was less toxic than the other two compounds. A test with embryos of the herring, Clupea harengus, and 2,4-dinitrophenol (Rosenthal and Stelzer, 1970) provided an adjusted LC50 value of 3,007 µg/l as compared to the value for the sheepshead minnow and the same chemical of 16,073 µg/l. The Final Fish Acute Values for these nitrophenols after adjustment for test methods and species sensitivity are 4,000 µg/l (4-nitrophenol), 1,900 µg/l (2,4-dinitrophenol) and 20,000 µg/l (2,4,6-trinitrophenol).

The mysid shrimp, Mysidopsis bahia, has also been exposed to the same nitrophenols (U.S. EPA, 1978) and, again, 2,4,6-trinitrophenol (96-hour LC50 of 16,686 µg/l) was less toxic than

4-nitrophenol (96-hour LC50 of 6,073 µg/l) and 2,4-dinitrophenol (96-hour LC50 of 4,108 µg/l) (Table 6). In general, the LC50 values for the mysid shrimp were about 2 to 4 times lower than comparable values for the sheepshead minnow. The Final Invertebrate Acute Values, and Final Acute Values since they are lower than those for fish, are 120, 84, and 340 µg/l for 4-nitrophenol, 2,4-dinitrophenol, and 2,4,6-trinitrophenol, respectively.

Chronic Toxicity

An embryo-larval test with the sheepshead minnow and 4-nitrophenol (U.S. EPA, 1978) is the only test with any nitrophenol that provides a chronic value. This concentration is 6,325 µg/l (Table 7) and is obtained by dividing the geometric mean of the highest no observed effect and lowest observed effect concentrations by two. The adverse effects observed were on hatching and survival. These results are not much lower than the unadjusted 96-hour LC50 value of 27,100 µg/l (Table 5) from the same study. The Final Fish Chronic Value derived after use of the species sensitivity factor (6.7) is 940 µg/l. This concentration is higher than the Final Acute Value (120 µg/l), because the latter is based on the more sensitive invertebrate species.

Plant Effects

The saltwater alga, Skeletonema costatum, is more sensitive to 4-nitrophenol with 96-hour EC50 values of 7,370 and 7,570 µg/l for inhibition of chlorophyll a and cell number production, respectively, than to 2,4-dinitrophenol and 2,4,6-trinitrophenol

(Table 8). The Final Plant Values for 4-nitrophenol, 2,4-dinitrophenol, and 2,4,6-trinitrophenol are 7,400, 93,000, and 63,000 µg/l, respectively.

Residues

No measured steady-state bioconcentration factors (BCFs) are available for any nitrophenol. BCFs can be estimated using the octanol-water partition coefficients of 32, 150, and 110 for 2,4-dinitrophenol, 2,4-dinitro-6-methylphenol, and 2,4,6-trinitrophenol, respectively. These coefficients are used to derive estimated BCFs of 8.1, 26, and 21 for 2,4-dinitrophenol, 2,4-dinitro-6-methylphenol and 2,4,6-trinitrophenol, respectively, for aquatic organisms that contain about 8 percent lipids. If it is known that the diet of the wildlife of concern contains a significantly different lipid content, appropriate adjustments in the estimated BCFs should be made. No estimates can be made for 2-nitrophenol and 4-nitrophenol.

Miscellaneous

The lethal threshold value after a 96-hour exposure of Atlantic salmon to 2,4-dinitrophenol (Zitko, 1976) is 700 µg/l (Table 9) which is lower than the Final Fish Acute Value (1,900 µg/l) but not the Final Acute Value (84 µg/l).

CRITERION FORMULATION

Saltwater-Aquatic Life

Summary of Available Data

The concentrations below have been rounded to two significant figures.

4-nitrophenol

Final Fish Acute Value = 4,000 µg/l

Final Invertebrate Acute Value = 120 µg/l

Final Acute Value = 120 µg/l

Final Fish Chronic Value = 940 µg/l

Final Invertebrate Chronic Value = not available

Final Plant Value = 7,400 µg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 940 µg/l

0.44 x Final Acute Value = 53 µg/l

2,4-dinitrophenol

Final Fish Acute Value = 1,900 µg/l

Final Invertebrate Acute Value = 84 µg/l

Final Acute Value = 84 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 93,000 µg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 93,000 µg/l

0.44 x Final Acute Value = 37 µg/l

2,4,6-trinitrophenol

Final Fish Acute Value = 20,000 µg/l

Final Invertebrate Acute Value = 340 µg/l

Final Acute Value = 340 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 63,000 µg/l

Residue Limited Toxicant Concentration = not available

Final Fish Chronic Value = 63,000 µg/l

0.44 x Final Acute Value = 150 µg/l

No saltwater criterion can be derived for most nitrophenols using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available.

Results obtained with 4-nitrophenol and saltwater organisms indicate how criteria may be estimated for other nitrophenols and saltwater organisms.

For 4-nitrophenol and saltwater organisms 0.44 times the Final Acute Value is less than the Final Chronic Value which is derived from results of an embryo-larval test with the sheepshead minnow. Therefore, it seems reasonable to estimate criteria for other nitrophenols and saltwater organisms using 0.44 times the Final Acute Value.

4-nitrophenol

The maximum concentration of 4-nitrophenol is the Final Acute Value of 120 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on saltwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 4-nitrophenol the criterion to protect saltwater aquatic life as derived using the Guidelines is 53 µg/l as a

24-hour average and the concentration should not exceed 120 µg/l at any time.

2,4-dinitrophenol

The maximum concentration of 2,4-dinitrophenol is the Final Acute Value of 84 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on saltwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2,4-dinitrophenol the criterion to protect saltwater aquatic life as derived using procedures other than the Guidelines is 37 µg/l as a 24-hour average and the concentration should not exceed 84 µg/l at any time.

2,4,6-trinitrophenol

The maximum concentration of 2,4,6-trinitrophenol is the Final Acute Value of 340 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on saltwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2,4,6-trinitrophenol the criterion to protect saltwater aquatic life as derived using procedures other than the Guidelines is 150 µg/l as a 24-hour average and the concentration should not exceed 340 µg/l at any time.

Table 5. Marine fish acute values for nitrophenols

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (µg/l)	Adjusted LC50 (µg/l)	Reference
Sheepshead minnow, *Cyprinodon variegatus*	S	U	4-nitrophenol	96	27,100	14,816	U.S. EPA, 1978
Sheepshead minnow, *Cyprinodon variegatus*	S	U	2,4-dinitrophenol	96	29,400	16,073	U.S. EPA, 1978
Herring (embryo), *Clupea harengus*	S	U	2,4-dinitrophenol	96	5,500	3,007	Rosenthal & Stelzer, 1970
Sheepshead minnow, *Cyprinodon variegatus*	S	U	2,4,6-trinitrophenol	96	134,000	73,258	U.S. EPA, 1978

* S = static

** U = unmeasured

Geometric mean of adjusted values:

4-nitrophenol = 14,816 µg/l $\frac{14,816}{3.7}$ = 4,000 µg/l

2,4-dinitrophenol = 6,928 µg/l $\frac{6,928}{3.7}$ = 1,900 µg/l

2,4,6-trinitrophenol = 73,258 µg/l $\frac{73,258}{3.7}$ = 20,000 µg/l

Table 6. Marine invertebrate acute values for nitrophenols (U.S. EPA, 1978)

Organism	Bioassay Method*	Test Conc. **	Chemical Description	Time (hrs)	LC50 (µg/l)	Adjusted LC50 (µg/l)
Mysid shrimp, *Mysidopsis bahia*	S	U	4-nitrophenol	96	7,170	6,073
Mysid shrimp, *Mysidopsis bahia*	S	U	2,4-dinitrophenol	96	4,850	4,108
Mysid shrimp, *Mysidopsis bahia*	S	U	2,4,6-trinitrophenol	96	19,700	16,686

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* S = static

** U = unmeasured

Geometric mean of adjusted values: 4-nitrophenol = 6,073 µg/l $\frac{6,073}{49}$ = 120 µg/l
2,4-dinitrophenol = 4,108 µg/l $\frac{4,108}{49}$ = 84 µg/l
2,4,6-trinitrophenol = 16,686 µg/l $\frac{16,686}{49}$ = 340 µg/l

Table 7. Marine fish chronic values for nitrophenols (U.S. EPA, 1978)

Organism	Test*	Limits (ug/l)	Chronic Value (ug/l)
Sheepshead minnow, *Cyprinodon variegatus*	E-L	10,000-16,000	6,325**

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* E-L = embryo-larval

** 4-nitrophenol

Geometric mean of chronic values = 6,325 ug/l $\frac{6,325}{6.7}$ = 940 ug/l

Lowest chronic value = 6,325 ug/l

Table 8. Marine plant effects for nitrophenols (U.S. EPA, 1978)

Organism	Effect	Concentration (ug/l)
Alga, *Skeletonema costatum*	96-hr EC50 Chlorophyll *a*	7,370 4-nitrophenol
Alga, *Skeletonema costatum*	96-hr EC50 cell number	7,570 4-nitrophenol
Alga, *Skeletonema costatum*	96-hr EC50 Chlorophyll *a*	93,200 2,4-dinitrophenol
Alga, *Skeletonema costatum*	96-hr EC50 cell number	98,700 2,4-dinitrophenol
Alga, *Skeletonema costatum*	96-hr EC50 Chlorophyll *a*	62,700 2,4,6-trinitrophenol
Alga, *Skeletonema costatum*	96-hr EC50 cell number	141,000 2,4,6-trinitrophenol

Lowest plant value: 4-nitrophenol = 7,370 µg/l

2,4-dinitrophenol = 93,200 µg/l

2,4,6-trinitrophenol = 62,700 µg/l

Table 9. Other marine data for nitrophenols

Organism	Test Duration	Effect	Result (µg/l)	Reference
		2,4-Dinitrophenol		
Atlantic salmon (juvenile), *Salmo salar*	96 hrs	Lethal threshold value	700	Zitko, 1976
Sea urchin (sperm), *Strongylocentrotus purpuratus*	1+ hrs	Inhibit respiration, motility	92,000	Bernstein, 1955
Sea urchin (embryo), *Pseudocentrotus depressus*	2 hrs	Abnormal cleavage	46,000	Kojima, 1960

NITROPHENOLS

REFERENCES

Bernstein, G.S. 1955. Effect of 2,4-dinitrophenol on sea urchin sperm. Proc. Soc. Exp. Biol. Med. 90: 28

Dedonder, A., and C.F. Van Sumere. 1971. The effect of phenolics and related compounds on the growth and respiration of Chlorella vulgaris. Z. Pflanzen. Physiol. 65: 70.

Flickinger, C.J. 1972. Influence of inhibitions of energy metabolism on the formation of Golgi bodies in Amebae. Exp. Cell Res. 73: 154.

Gersdorff, W.A. 1939. Effect of the introduction of the nitro group into the phenol molecule on toxicity to goldfish. Jour. Cell. Comp. Physiol. 14: 61.

Huang, J., and E. Gloyna. 1967. Effects of toxic organics on photosynthetic reoxygenation. Environ. Health Eng. Res. Lab. PB 216-749.

Kojima, M.K. 1960. The effect of DNP and NaN_3 on fertilized eggs of the sea urchin with special reference to the induction of the abnormal cleavage. Embryologia 5: 71.

Kopperman, H.L., et al. 1974. Aqueous chlorination and ozonation studies. I. Structure-toxicity correlations of phenolic compounds to Daphnia magna. Chem. Biol. Interact. 9: 245.

Lammering, M.W., and N.C. Burbank. 1960. The toxicity of phenol, o-chlorophenol and o-nitrophenol to bluegill sunfish. Eng. Bull. Purdue Univ. Engin Ext. Serv. 106: 541.

Lewis, E.J.C., and E. Frieden. 1959. Biochemistry of amphibian metamorphosis: effect of triiodothyronine, thyroxin, and dinitrophenol on the respiration of the tadpole. Endocrinology 65: 273.

Marcus, M., and A.M. Mayer. 1963. Flagellar movement in Chlamydomonas snowiae and its inhibition by ATP and dinitrophenol. In Studies on microalgae and photosynthetic bacteria. Jap. Soc. Plant Physiol. University of Tokyo Press, Tokyo. Japan.

Phipps, G.L., et al. The acute toxicity of phenol and substituted phenols to the fathead minnow. (Manuscript).

Rosenthal, H., and R. Stelzer. 1970. Wirkungen von 2,4- und 2,5-dinitrophenol auf die Embryonalentwicklung des Herings Clupea harengus. Mar. Biol. 5: 325.

Sanders, H.O., and O.B. Cope. 1968. The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. 13: 112.

Simon, E.W., and G.E. Blackman. 1953. Studies in the principles of phytotoxicity. IV. The effects of the degree of nitration on the toxicity of phenol and other substituted benzenes. Jour. Exp. Bot. 4: 235.

U.S. Environmental Protection Agency. 1978. In-depth studies on health and environmental impacts of selected water pollutants. Contract No. 68-01-4646.

Zitko, V., et al. 1976. Toxicity of alkyldinitrophenols to some aquatic organisms. Bull. Environ. Contam. Toxicol. 16: 508.

MONONITROPHENOLS

Mammalian Toxicology and Human Health Effects

EXPOSURE

Introduction

Mononitrophenol has three isomeric forms, distinguished by the position of the nitro group on the phenolic ring. Three isomeric forms are possible, namely 2-nitrophenol, 3-nitrophenol, and 4-nitrophenol. The compounds are also commonly referred to as o-nitrophenol, m-nitrophenol, and p-nitrophenol, respectively.

Commercial synthesis of 2-nitrophenol and 4-nitrophenol is accomplished through the hydrolysis of the appropriate chloronitrobenzene isomers with aqueous sodium hydroxide at elevated temperatures (Howard, et al. 1976). Production of 3-nitrophenol is achieved through the diazotization and hydrolysis of m-nitroaniline (Matsuguma, 1967). The mononitrophenol isomers are used in the United States primarily as intermediates for the production of dyes, pigments, pharmaceuticals, rubber chemicals, lumber preservatives, photographic chemicals and pesticidal and fungicidal agents (U.S. Int. Trade Comm. 1976). As a result of this use pattern, the major source for environmental release of mononitrophenols is likely to be from production plants and chemical firms where the compounds are used as intermediates. The mononitrophenols may also be inadvertently produced via microbial or photodegradation of pesticides which contain mononitrophenol moieties. Aproximately 10 to 15 million pounds of 2-nitrophenol are produced annually (Howard, et al. 1976) for uses including synthesis of o-aminophenol, o-nitroanisole, and other dye stuffs (Matsuguma, 1967; Howard, et al. 1976). Although production figures for 3-nitrophenol are not available, Hoecker, et al. (1977) estimate that production is less than one million pounds annually. 3-nitrophenol is used in the manufacture of dye intermediates such as anisidine and m-aminophenol (Kouris and Northcott, 1963; Matsuguma, 1967). 4-nitrophenol is probably the most important of the mononitrophenols in terms of quantities used and potential environmental contamination. Demand for 4-nitrophenol was 35,000,000 pounds in 1976 and production is projected to increase to 41,000,000 pounds by 1980 (Chem. Market. Reporter, 1976). Most of the 4-nitrophenol produced (87 percent) is used in the manufacture of ethyl and methyl parathions. Other uses (13 percent) include the manufacture of dye-stuffs and n-acetyl-p-aminophenol (APAP) and leather treatments. A possible source of human exposure to 4-nitrophenol is as a result of microbial or photodegradation of the parathions. *In vivo* production of 4-nitrophenol following absorption of parathion or other pesticides by humans is another possible source of human exposure.

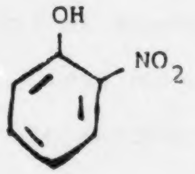
Physical and chemical properties of the mononitrophenols are summarized in Table 1.

TABLE 1

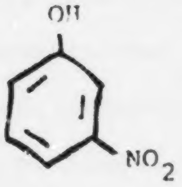
Properties of Mononitrophenols

	2-Nitrophenol	3-Nitrophenol	4-Nitrophenol
Formula	$C_6H_5NO_3$	$C_6H_5NO_3$	$C_6H_5NO_3$
Molecular Weight	139.11	139.11	139.11
Melting Point (°C)	44-45	97	113-114
Boiling Point	214-216	194	279
Density	1.485	1.485	1.479
Water Solubility (g/l)	0x3.2 at 38°C 1x0.8 at 100°C	1x3.5 at 25°C 13x3.0 at 90°C	0x8.04 at 15°C 1x6.0 at 25°C
Vapor Pressure	1 mm Hg at 49.3°C		
Ka	$7.5x10^{-8}$	$5.3x10^{-9}$	$7x10^{-8}$

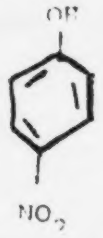
Mononitrophenols



2-nitrophenol



3-nitrophenol



4-nitrophenol

Ingestion from Water

Monitoring data on the presence of mononitrophenols in water are scant in the literature. Potential point sources for mononitrophenol contamination of water include industrial concerns engaged in the manufacture of these compounds or their usage as intermediates in chemical synthesis. Trifunovic, et al. (1971) detected unspecified levels of 4-nitrophenol in waste effluents from a parathion manufacturing plant. Webb, et al. (1973) reported a 4-nitrophenol level of 1.4 mg/l in the waste lagoon water of a chemical plant. Burnham, et al. (1972) detected 4-nitrophenol at levels of 0.2 mg/l in the potable water supply of Ames, Iowa. The source of the contamination was believed to be residues from a coal gas plant which ceased operation around 1930. 2-Nitrophenol was detected at unidentified levels in two river water samples and in 4 samples of chemical plant effluent, and 3-nitrophenol was found in one chemical plant effluent sample (U.S. EPA, 1976). Systematic monitoring for mononitrophenols in the environment has not been done. It is reasonable to assume that measureable (although perhaps transient) levels of the mononitrophenols may be present in localized areas where organophosphate pesticides are in use.

Little data is available regarding the breakdown of mononitrophenols by natural communities of microorganisms. Alexander and Lustigman (1966) studied the degradation of mononitrophenols by a mixed population of soil microorganisms. The inoculum was derived from a suspension of Niagra silt loam soil. Their results indicated that 2-nitrophenol was more resistant to degradation than either 3-nitrophenol or 4-nitrophenol. Utilizing the absorbancy of small soil inoculums to estimate the loss of mononitrophenol, 3-nitrophenol was found to degrade completely within a 4-day period. 4-nitrophenol degraded fully within a 16-day period while 2-nitrophenol resisted degradation over a 64-day period.

Brebion, et al. (1967) examined the ability of microorganisms derived from soil, water, or mud, and grown on a porous mineral bed to attack 4-nitrophenol. The bacteria were cultivated on a mineral nutrient solution to which nitrophenols were added as the sole source of carbon. The experimental findings revealed no significant removal of the compound under these conditions.

In contrast to these reports, a number of investigators have found that the mononitrophenols are readily and rapidly degraded by acclimated populations of microorganisms. Tabak, et al. (1964) studied the ability of acclimated cultures derived from garden soil, compost, and river mud to degrade the mononitrophenols. Phenol-adapted bacteria derived from these sources were found to readily degrade all three mononitrophenol isomers. Ninety-five percent degradation (measured spectrophotometrically) occurred within three to six days. Pitter (1976) reported greater than 95 percent degradation of the three mononitrophenol isomers in an acclimated sludge system. The nitrophenols served as the sole source of organic carbon and degradation was complete within 120 hours.

A recent study (Haller, 1978) reports on the ability of unacclimated microorganisms to degrade the mononitrophenols. Either sludge obtained from the primary settling tank of the city of Ithaca, N.Y. wastewater treatment plant, or a Windsor or 4-nitrophenol. Utilizing the absorbancy of small soil inoculums to estimate the loss of mononitrophenol, 3-nitrophenol was found to degrade completely within a 4-day period. 4-nitrophenol degraded fully within a 16-day period while 2-nitrophenol resisted degradation over a 64-day period.

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A recent study (Haller, 1978) reports on the ability of unacclimated microorganisms to degrade the mononitrophenols. Either sludge obtained from the primary settling tank of the city of Ithaca, N.Y. wastewater treatment plant, or a Windsor loamy fine sand soil were used as the source of the inoculum. 2-Nitro, 3-nitro, and 4-nitrophenol (16 mg/l) were completely degraded in three to five days by the sludge system. Soil inocula degraded 16 mg/l of 3-nitrophenol in three to five days while a similar concentration of 2-nitrophenol and 4-nitrophenol required 7 to 14 days for complete degradation.

Although definitive conclusions cannot be derived from this limited number of studies, it appears that the mononitrophenols are readily and rapidly degraded by microbial population present in the environment.

Ingestion from Foods

No data were found demonstrating the presence of mononitrophenols in food. One possible source of mononitrophenol exposure for humans is through the food chain as a result of the ingestion of food crops contaminated with pesticides containing the nitrophenol moiety. The production of 4-nitrophenol by microbial metabolism of parathion is well documented (Munnecke and Hsieh, 1974, 1976; Siddaramappa, et al. 1973; Sethunathan and Yoshida, 1973; Katan and Lichtenstein, 1977; Sethunathan, 1973). Microbial metabolism of fluoridifen (p-nitrophenyl, a, a, a-trifluoro-2-nitro-p-tolyl ether) results in the intermediate formation of 4-nitrophenol (Tewfik and Hamdi, 1975). The major degradation product of fluoridifen following uptake by peanut seedling roots was 4-nitrophenol (Eastin, 1971). 4-nitrophenol was also detected in soybean roots following absorption of fluoridifen (Rogers, 1971). Photodecomposition of the herbicide nitrofen (2,4-dichlorphenyl p-nitrophenyl ether) in aqueous suspensions under sunlight or simulated sunlight is characterized by rapid cleavage of the ether linkage to form 2, 4-dichlorophenol and 4-nitrophenol (Nakagawa and Crosby, 1974). El-Refai and Hopkins (1966) have investigated the metabolic fate of parathion following foliar application or root absorption by bean plants, Phaseolus vulgaris. Detectable amounts of 4-nitrophenol were found in chloroform rinses of parathion treated leaves after four days.

In another experiment, analysis of nutrient solutions containing parathion in which plants were grown for root absorption studies revealed 4-nitrophenol, paraoxon, and traces of degradation products. Since these compounds were also detected in control solutions which did not contain plants, the authors concluded that possible photochemical oxidation processes had occurred in the aqueous medium. The authors believed that the 4-nitrophenol detected following foliar application of parathion was due to photochemical processes. 4-nitrophenol was not detected in bean plants following injection of parathion directly into the stems of bean plants (El-Refai and Hopkins, 1966).

4-nitrophenol has also been detected as a photoalteration product of parathion following application to cotton plants (Joiner and Baetcke, 1973).

Archer (1974) has examined the dissipation of parathion and its metabolites from field spinach. Field plots were sprayed with either 0.5 or one pound of active parathion/acre. Application recommendations for parathion are: not less than 14 days before harvest at the rate of 0.5 pounds of active ingredient/acre. Spinach samples were analyzed daily for parathion residues and a number of known metabolites including 4-nitrophenol. Levels of 4-nitrophenol in the treated spinach (calculated on a fresh weight basis) are presented in Table 2. Unsprayed spinach control samples taken prior to any spray treatments contained 95 µg/kg 4-nitrophenol. The source of these residues was not determined. The effects of washing or blanching following harvest on the levels of 4-nitrophenol in human food crops are unknown.

4-nitrophenol has been detected in human urine. The National Monitoring Program for Pesticides is collaborating with the U.S.Public Health Service in a three-year study to assess the exposure of the general population of the U.S. through analysis of human urine for residues of selected pesticides and their specific metabolites (Kutz, et al. 1978). Based on the analysis of 416 samples collected from the general population, 4-nitrophenol is detected in 1.7 percent of the population. A mean urine level of 10.0 µg/l with a maximum value of 113.0 µg/l was reported. It is important to note that 4-nitrophenol residues in the urine need not (and probably do not) reflect exposure to 4-nitrophenol itself. Mononitrophenols are readily formed in vivo following exposure to a number of widely used pesticides.

Kutz, et al. (1978) considered exposure to methyl and ethyl parathion as the origin of the urinary 4-nitrophenol detected in their survey. However, if it is assumed that the reported urinary residues of 4-nitrophenol reflect direct exposure to the 4-nitrophenol, a pharmacokinetic estimate of

TABLE 2

Levels of 4-Nitrophenol Following Aplication of Parathion to Field Spinach at Two Different Application Rates[a]

SAMPLE DAY	4 Nitrophenol Residue (μg/kg) [b] [c]	
	0.5 lb. Parathion/Acre	1.0 lb. Parathion/Acre
1	172	453
2	88	305
3	73	240
4	76	188
5	73	136
6	72	216
7	35	117
8	40	18
9	34	19
10	34	18
11	31	18
12	38	22
13	28	16
14	33	19

[a] Source: Modified from Archer, 1974.

[b] Calculated on a fresh weight basis. Percent moisture from 86.4 to 89.2.

[c] Unsprayed spinach control samples taken prior to any spray treatments contained 95 μg/kg.

exposure can be made. Assuming that the exposure to nitrophenol is steady-state, that 100 percent of the absorbed nitrophenol is excreted in the urine, and that the average urine void is 1.4 l/day per 70 kg person, initial exposure levels can be estimated from residual levels found in urine. For example, the exposure level leading to the 1.7 µg/l residue can be calculated as follows:

$$\text{Exposure} = \frac{(10.0\ \mu\text{g nitrophenol/l})\ (1.4\ \text{l of urine/day})}{70\ \text{kg man}} = 0.2\ \mu\text{g/kg/day}$$

A similar calculation using the maximum urine residue level observed by Kutz, et al. (1978) (113 µg/l) gives an exposure of 2.26 µg/kg/day.

Knowles, et al. (1975) have demonstrated the production of a wide number of mono-nitrophenols including 2-nitrophenol in a model system simulating gastric digestion of smoked bacon. These studies, utilizing nitrosated liquid smoke, were conducted under conditions favorable to nitrosation, and since the temperature, pH, and duration employed approximated those encountered during gastric digestion, their results indicated that nitrosation of phenols in smoked bacon may occur in the stomach with resultant production of 2-nitrophenol.

Mononitrophenols may also be formed in vivo via metabolic degradation of pesticides such as parathion by humans.

Excretion of 4-nitrophenol, a metabolite of the organophosphorus pesticides, parathion, methylparathion, EPN, and dicapthon is a good indicator of human exposure to these pesticides (Wolfe, et al. 1970; Broadway and Shafik, 1973; Elliott, et al. 1960; Roan, et al. 1969). 4-nitrophenol has also been detected as a urinary metabolite of nitrobenzene in humans (Myslak, et al. 1971).

A bioconcentration factor (BCF) relates the concentration of a chemical in water to the concentration in aquatic organisms, but BCF's are not available for the edible portions of all four major groups of aquatic organisms consumed in the United States. Since data indicate that the BCF for lipid-soluble compounds is proportional to percent lipids, BCF's can be adjusted to edible portions using data on percent lipids and the amounts of various species consumed by Americans. A recent survey on fish and shellfish consumption in the United States (Cordle, et al. 1978) found that the per capita consumption is 18.7 g/day. From the data on the nineteen major species identified in the survey and data on the fat content of the edible portion of these species (Sidwell, et al. 1974), the relative consumption of the four major groups and the weighted average percent lipids for each group can be calculated:

Group	Consumption (Percent)	Weighted Average Percent Lipids
Freshwater fishes	12	4.8
Saltwater fishes	61	2.3
Saltwater molluscs	9	1.2
Saltwater decapods	18	1.2

Using the percentages for consumption and lipids for each of these groups, the weighted average percent lipids is 2.3 for consumed fish and shellfish.

No measured steady-state bioconcentration factor (BCF) is available for any nitrophenol, but the equation "Log BCF = 0.76 Log P - 0.23" can be used (Veith, et al., Manuscript) to estimate the BCF for aquatic organisms that contain about eight percent lipids from the octanol-water partition coefficient (P). An adjustment factor of 2.3/8.0 = 0.2875 can be used to adjust the estimated BCF from the 8.0 percent lipids on which the equation is based to the 2.3 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for edible portion of all aquatic organisms consumed by Americans can be calculated.

Table 2A

Compound	P	BCF	Weighted BCF
2-nitrophenol	62	14	4.0
4-nitrophenol	81	17	4.9
2,4-dinitrophenol	32	8.2	2.4
2,4,6-trinitrophenol	110	21	6.0
4,6-dinitro-o-cresol	150	26	7.5

Inhalation

No quantitative data were found regarding the presence of mononitrophenols in air. Lao, et al. (1973) discussed the application of a gas chromatograph quadrapole mass spectrometer-data processor combination for routine analysis of air pollutants. During a sample run of urban ambient particulate matter (location not designated) these investigators identified the presence of 4-nitrophenol as well as a large number of other air pollutants. No quantitative data were provided, however. Ambient air levels of 4-nitrophenol in a Boeing plant where the compound was used for the preservation of the cork surfaces of the Minuteman Missile were equal to or less than 0.05 mg/m^3 of air (Butler and Bodner, 1973).

4-nitrophenol may be produced in the atmosphere through the photochemical reaction between benzene and nitrogen monoxide. Nojima, et al. (1975) irradiated a combination of benzene vapor and nitrogen monoxide gas for five hours with a xenon lamp and characterized the resulting photochemical products. The production of nitrobenzene, 2-nitrophenol, 4-nitrophenol, 2, 4-dinitrophenol and 2, 6-dinitrophenol was described by the authors. Identity of the compounds was confirmed using thin layer chromatography, gas chromatography, gas-chromatography-mass spectrometry, and infrared spectrometry. The authors suggested that these nitro compounds may be the cause of the characteristic symptoms of seriously stricken victims of photochemical smog in Japan including: headache, breathing difficulties, vomiting, rise in body temperature, and numbness in the extremities.

In a second paper (Nojima, et al. 1976), the photochemical reaction of toluene with nitrogen monoxide was investigated. It was felt that the products of photochemical reaction of toluene with nitrogen monoxide might be more important in the production of photochemical smog since the concentration of toluene in urban air is higher than that of benzene. Compounds produced as a result of this reaction included o-cresol, m-nitrotoluene, 4-nitrophenol, 2-methyl-6-nitrophenol, 3-methyl-4-nitrophenol, 2-methyl-4-nitrophenol and 2-methyl- 4,6-dinitrophenol. These compounds were identified by gas chromatography-mass spectrometry. In another experiment, the investigators examined the organic compounds present in rain. An analysis of rainwater yielded 4-nitrophenol, 2-methyl-6-nitrophenol, and 2-methyl-4-nitrophenol. The authors suggested that the nitrophenols produced by the photochemical reactions described above, dissolve in rain. It seems likely that in areas where severe photochemical smog exists, humans may be exposed to substantial levels of mononitrophenols. However, it is impossible to estimate the levels at which humans are exposed to these compounds via inhalation, based on available data.

Dermal

Roberts, et al. (1977) used human autopsy skin epidermal membranes in an in vitro system to determine the permeability of human skin to various compounds. Both 3-nitrophenol and 4-nitrophenol were shown to permeate the skin and to produce damage at threshold concentrations of 0.8 and 0.9 percent (w/v), respectively. According to Patty (1963), 2-nitrophenol may be absorbed through the intact skin. No information on possible human dermal exposure to the mononitrophenols was found.

PHARMACOKINETICS

Absorption and Distribution

Data specific to the absorption and tissue distribution of the mononitrophenols were not available. It is reasonable to assume, based on the rapid urinary elimination of the mononitrophenols, that the compounds may be resticted primarily to the blood and urine following absorption by humans.

Metabolism

Metabolism of the mononitrophenols probably occurs via one of three mechanisms in humans. The major route of mononitrophenol metabolism is undoubtedly conjugation and the resultant formation of either glucuronide or sulfate conjugates. Other possible routes of metabolism include reduction to amino compounds or oxidation to dihydric-nitrophenols.

Sulfate and glucuronide conjugative processes are two of the major detoxification mechanisms in many species, including mammals (Quebbemann and Anders, 1973). In recent years, 4-nitrophenol has been used as a preferred substrate for biochemical analysis of the glucuronidation reactions in a wide number of species (Aitio, 1973; Sanchez and Tephly, 1974; Ranklin, 1974; Heenan and Smith, 1974; Yang and Wilkinson, 1971). This usage reflects the simple techniques available for quantitating the disappearance of 4-nitrophenol and the synthesis of the glucuronide conjugate. The relevance of many of these in vitro studies towards an assessment of the metabolic fate of the mononitrophenols in humans is questionable; thus only those in vivo studies with direct relevance to the metabolic fate of the mononitrophenols in humans or experimental animals are discussed here.

It has been known for some time that levels of the mixed function oxidases and the enzymes responsible for conjugation of many compounds are generally highest in the mammalian liver. Litterst, et al. (1975) assayed liver, lung and kidney from rat, mouse, rabbit, hamster and guinea pig for standard microsomal and soluble fraction enzymes involved in drug biotransformation. These studies included an analysis of glucuronide conjugation of 4-nitrophenol by these tissues. For all species, liver was the most active organ. Kidney and lung activities were usually 15 to 40 percent of that found in liver with kidney slightly more active than lung. UDP-glucuronyl- transferase activity toward the acceptor 4-nitrophenol was higher in hamster and rabbit than other species.

Conjugation activity need not be a constant even within the same species, however. Pulkkinen (1966b) noted that sulfate conjugation of 4-nitrophenol is decreased during pregnancy in rabbits. The author suggested that large amounts of estrogens may cause more protein binding, thus inhibiting the reaction. In another study (Pulkkinen, 1966a) it was noted that conjugation capacity increases with age in the rat, guinea pig and man. The human fetus does not have a very high capacity to form sulfate or glucuronide conjugates of mononitrophenols or other compounds. As age increases, so does conjugation capacity. In addition, Moldeus, et al. (1976) noted that the relative rate of glucuronide versus sulfate conjugation of 4-nitrophenol may depend on the levels of substrate present. In in vitro tests utilizing isolated rat liver cells, the investigators noted that at 4-nitrophenol concentrations of 25 μM the rate of glucuronide conjugation was low and over 75 percent of the conjugation products were found to be sulfates. The glucuronidation increased more rapidly than did the sulfate conjugation with increasing substrate conjugation. At 250 μM 4-nitrophenol, sulfate conjugation was inhibited almost completely and more than 95 percent of the conjugates formed were found to be glucuronides.

Robinson, et al. (1951) studied the metabolic detoxification of the mononitrophenol isomers in rabbits. They showed that, with doses of 0.2 to 0.3 g/kg, conjugation in vitro with glucuronic and sulfuric acids was almost complete.

Only small amounts (less than one percent) of the unchanged free phenol were excreted. With all three of the mononitrophenol isomers, the major conjugation product was nitrophenyl-glucuronide, which accounted for about 70 percent of the dose. The corresponding sulfate conjugates were also excreted. Reduction of the nitrophenols occurred to a small extent, the reduction of the 4- isomer being slightly greater than that of the 2- and 3- isomers. The mononitrophenols were also shown to undergo oxidation to a very small extent (less than one percent). 2-nitrophenol yields traces of nitroquinol; 3-nitrophenol yields nitroquinol and 4-nitrocatechol; and 4-nitrophenol yields 4-nitrocatechol.

A summary of the metabolism of the mononitrophenols is shown in Table 3. No data directly addressing the metabolic fate of the mononitrophenols in humans are available. However, it is expected that following exposure to the mononitrophenols humans will rapidly excrete both glucuronide and sulfate conjugates in the urine.

Excretion

Data directly addressing the excretion of the mononitrophenols following exposure of humans were not found in the literature. However, excretion patterns for 4-nitrophenol following human exposure to parathion may shed some light on their elimination kinetics. Arterberry, et al. (1961) studied the pharmaco-dynamics of 4-nitrophenol excretion following exposure to parathion. They noted that the excretion of 4-nitrophenol in the urine was quite rapid "as might be

TABLE 3

Urinary Metabolites of Mononitrophenols in Rabbits[a]

Nitrophenol	Percentage of Dose Excreted as					
	Nitro Compounds (N)	Amino Compounds (A)	(N + A)[b]	Glucuronides (G)	Ethereal Sulphates (E)	(G + E)[b]
2-Nitrophenol	82	3	85	71	11	82
3-Nitrophenol	74	10	84	78	19	98
4-Nitrophenol	87	14	101	65	16	81

[a] Source: Modified from Robinson, et al. 1951.

[b] (N + A) should be roughly equal to (G + E) since the amounts of free phenols excreted were very small. Both glucuronides and ethereal sulphates include nitro and amino conjugates.

expected in the case of a water-soluble metabolite of a substance which is quickly broken down by the animal organism." 4-nitrophenol usually had disappeared from the urine within about 48 hours after cessation of exposure. In a similar study of orchard spray-men involved in the application of parathion, Wolfe, et al. (1970) noted that urinary levels of 4-nitrophenol rose promptly in response to parathion exposure by spray-men and returned to the nondetectable level after several days. Myslak, et al. (1971) reported on the excretion of 4-nitrophenol from a 19-year-old female subject following a suicidal oral dose of nitrobenzene. Large quantities of 4-nitrophenol and 4-aminophenol were detected in the urine. Elimination of 4-nitrophenol in the urine was expressed by the equation $V_t/V_o = e^{-0.008t}$ where V^o and V^t denote the excretion rate at the interval time 0 and t measured in hours. The half-life for excretion corresponded to about 84 hours.

Shafik, et al. (1973) studied the urinary excretion of 4-nitrophenol following administration of the pesticide EPN. Following oral administration of the pesticide for three days, animals were maintained and urine samples collected at 24-hour intervals. Three days were required for complete excretion of 4-nitrophenol under these conditions. The foregoing studies indicate that 4-nitrophenol is rapidly excreted following its production in vivo from other organic compounds.

Only one study was found that examined excretion of 4-nitrophenol following direct administration of the compound. Lawford, et al. (1954) studied the elimination of various nitrophenolic compounds from the blood of experimental animals. Elimination of 4-nitrophenol by the monkey following oral and intraperitoneal doses of 20 mg/kg body weight was complete within five hours. Elimination by mice, rats, rabbits, and guinea pigs was also rapid. Most doses were eliminated completely from the blood within two hours of administration. Experimental animals eliminated 4-nitrophenol from the blood in the following descending order of efficiency: mouse, rabbit, guinea pig, rat, and monkey.

In summary, the available data indicate that the mononitrophenols are excreted rapidly via the urinary route and that total elimination is likely not to exceed one week. The mononitrophenols are highly water soluble and accumulation or bioconcentration in various tissues is not expected to occur to a large extent. However, much more data are needed to precisely define the transport distribution and elimination of these compounds in humans.

EFFECTS

Threshold concentrations for odor, taste, and color for 2-nitro, 3-nitro, and 4-nitrophenol in reservoir water have been reported in an abstract of a paper from the Russian literature (Makhinya, 1964). Reported threshold concentrations for 2-nitrophenol were 3.83 mg/l for odor, 8.6 mg/l for taste, and 0.6 mg/l for color. Concentrations for 4-nitrophenol were 58.3, 43.4, and 0.24 mg/l for odor, taste, and color, respectively. The values for 3-nitrophenol were given as 389, 164.5 and 26.3 mg/l. Acceptability thresholds from the standpoint of human consumption were not reported by these investigators.

Acute, Sub-acute, and Chronic Toxicity

Known effects of 4-nitrophenol demonstrated in animal experiments are methemoglobinemia, shortness of breath, and initial stimulation followed by progressive depression (von Oettingen, 1949).

Acute toxicity information for the mononitrophenol isomers has been compiled and presented as Table 4. 4-nitrophenol is the most toxic of the mononitrophenols followed by 3-nitrophenol and 2-nitrophenol in relative toxicity. Toxicological symptoms of mononitrophenol poisoning have not been well described in the literature. Sax (1968) noted that 2-nitrophenol exposure produced kidney and liver injury in experimental animals. Methemoglobin formation as a result of mononitrophenol exposure has been reported (Patty, 1963). Grant (1959), however, was unable to detect methemoglobin formation after oral administration of 3-nitro and 4-nitrophenol to rats. Small inconstant amounts of methemoglobin were formed with 3-nitrophenol administration. Smith, et al. (1967) were able to show that the reduction products of mononitrophenols, 2- and 4-aminophenol, would produce methemoglobin in female mice. Methemoglobin formation, therefore, may depend on the capacity of the organism to reduce the mononitrophenols. As mentioned in the metabolism section of

TABLE 4

Acute Toxicity of Mononitrophenol Isomers

Species	Dose (mg/kg)	Route of Administration	Effects	References
		2-Nitrophenol		
Frog	300	S.C.	Lethal Dose	Spector, 1956
Mouse	600	I.M.	Lethal Dose	Spector, 1956
Rabbit	1700	S.C.	Lethal Dose	Spector, 1956
Cat	600	S.C.	Lethal Dose	Spector, 1956
Dog	100	I.V.	Lethal Dose	Spector, 1956
Rat	2830	Oral	LD 50	Vernot, et al. 1977
Mouse	1300	Oral	LD 50	Vernot, et al. 1977
Guinea Pig	900	S.C.	Lethal Dose	Spector, 1956
		3-Nitrophenol		
Dog	83	I.V.	Minimum Lethal Dose	Spector, 1956
Rat	930	Oral	LD 50	Vernot, et al. 1977
Mouse	1410	Oral	LD 50	Vernot, et al. 1977
		4-Nitrophenol		
Frog	50	S.C.	Minimum Lethal Dose	Spector, 1956
Rabbit	600	S.C.	Minimum Lethal Dose	Spector, 1956
Cat	197	S.C.	Minimum Lethal Dose	Spector, 1956
Dog	10	I.V.	Lethal Dose	Spector, 1956
Rat	620	Oral	LD 50	Vernot, et al. 1977
Mouse	470	Oral	LD 50	Vernot, et al. 1977
Rat	350	Oral	LD 50	Fairchild, 1977

this document, reduction of the nitrophenols does not normally occur to any large extent.

Ogino and Yasukura (1957) reported the development of cataracts in vitamin C deficient guinea pigs following administration of 4-nitrophenol. Cataracts developed in two of three guinea pigs on days 7 and 11 following daily intraperitoneal administration of 8.3 to 12.5 mg 4-nitrophenol/kg body weight. Subchronic administration of 4-nitrophenol over a 20-day test period produced cataracts while 2- and 3-nitrophenol did not. The authors concluded that the *para*-positioning of the hydroxyl and nitro groups is necessary for cataract induction.

Several deficiencies in this study preclude definitive conclusions on the cataractogenic properties of 4-nitrophenol. The investigators failed to report results on control animals, either totally untreated or treated with the nitrophenols and a vitamin C supplement. Thus, it is possible, based on the results reported, to conclude that vitamin C deficiency itself caused cataracts in some of the animals tested. The small size of the experimental groups (three animals/test compound) also make definitive conclusions difficult. The reported conclusions must be taken with considerable caution based on the above considerations.

In contrast, Dietrich and Beutner (1946) found 2-nitro and 4-nitrophenol to be devoid of cataract-forming activity in seven-day-old chicks. Animals were fed on a commercial brand of chick food containing 0.25 percent nitrophenol.

Although cataracts developed rapidly (within 24 to 48 hours) when the animals were fed 2, 4-dinitrophenol, no cataracts developed within a three-week period when animals were fed the mononitrophenol isomers. The capacity for cataract formation in humans following mononitrophenol exposure is unclear.

Both 2-nitro and 4-nitrophenol have been shown to inhibit porcine heart malate dehydrogenase in vitro (Wedding, et al. 1967). The compounds acted as competitive inhibitors for NAD in the forward direction of the enzymatic reaction. The clinical significance of these findings is unknown.

The ventilatory effects of the mononitrophenols have been examined in anesthetized rats (Grant, 1959). Test compounds were administered by stomach tube: 2-nitrophenol, 60 to 120 mg; 3-nitrophenol, 20 to 45 mg; 4-nitrophenol, 7 to 12 mg. Significant increases in respiratory volume ranging from 15 to 30 percent were reported in these experiments.

Neither carbon dioxide output nor oxygen uptake were affected by sublethal doses of 2-nitrophenol in rats (Cameron, 1958). In contrast, oxygen uptake was decreased in 3-nitrophenol-treated rats while carbon dioxide output was increased following 4-nitrophenol administration. Rectal temperature was depressed in rats receiving any of the three isomers. These results suggest that mononitrophenol isomers are not potent uncouplers of oxidative phosphorylation, in contrast to the chemically similar compound 2, 4-dinitrophenol.

Although the mechanism of toxic action of the mononitrophenols is not well understood, the following studies suggest that an action directly on cell membranes may occur. 3-nitrophenol binds readily to red blood cell (RBC) membranes. Expansion of RBC ghosts occurs following nitrophenol treatments, as measured by the resistance of such ghosts to hemolysis (Machleidt, et al. 1972). 2-nitrophenol and 4-nitrophenol inhibit chloride transport in red blood cells (a metabolism independent process) suggesting a direct action on the cell membrane (Motais, et al. 1978). Further information on the acute or chronic toxicity of the mononitrophenols to humans was not found.

The National Institute for Occupational Safety and Health recently undertook a health hazard evaluation determination at the request of an employee of the Boeing Company who had routinely handled 4-nitrophenol (Butler and Bodner, 1973). A 15 percent solution of 4-nitrophenol and methylphenol is painted on the exposed cork surfaces of the Minuteman Missile before arrival at the assembly plant. If the surface is damaged in transit it is necessary to apply small amounts of the 4-nitrophenol solution to the repaired areas of cork. The worker in question was engaged in such touch-up operations. Workers routinely wear an organic vapor cartridge respirator, a face shield, cotton gloves, rubber gloves, and are completely covered with protective clothing. The employee complained of fatigue, joint pain, abdominal cramps and diarrhea, and attributed these symptoms to his exposure to both the treating solution and the dried cork impregnated with 4-nitrophenol during his work as a mechanic. Medical examination failed to detect 4-nitrophenol in the urine but revealed a complete absence of the immunoglobins IgA and IgD in the employee. Based on medical judgement and the existing literature, the study concluded that the employee's condition stemmed from the lack of IgA and IgD and that this deficiency was not caused by exposure to 4-nitrophenol.

Gabor, et al. (1960; cited in Howard, et al. 1976) reported a unique effect of 2-nitrophenol on blood platelet levels. When 31 rats were administered 2-nitrophenol by intraperitoneal injection at 1 mg/kg body weight, the platelet count increased significantly. Even at doses of 0.1 mg/kg a similar effect was produced. Administration of 3-nitro or 4-nitrophenol did not produce a rise in platelet levels. Additional data are not available to explain this unique phenomenon nor is the clinical significance of these findings known.

A report from the Russian literature (Makhinya, 1969) reports that 2-nitro, 3-nitro, and 4-nitrophenol possess distinct cumulative properties. Chronic administration of any of the mononitrophenols to mammals caused alterations of neurohumoral regulation and pathological changes including colitis, enteritis, hepatitis, gastritis, hyperplasia of the spleen, and neuritis. Limiting doses for the disruption of conditioned reflex activity were established as .003 mg/kg (equivalent to .006 mg/l of water) for 2-nitrophenol and 3-nitrophenol and .00125 mg/kg (equivalent to .0025 mg/l of water) for 4-nitrophenol. Unfortunately a report of this study was available in abstract form only. Details of the experiment including animal species, mode of administration, duration of the treatment, and a good description of the observed biological effects, were not reported. The results must be considered questionable until an evaluation of the experimental protocol becomes available.

Synergism and/or Antagonism

Only one report was found dealing with possible synergistic effects of the mononitrophenols. Cairns, et al. (1976) studied the effects of a sublethal exposure to zinc and subsequent toxicity of 4-nitrophenol to snails, *Goniobasis livescens*. Snails were exposed for 96 hours to two sublethal concentrations of zinc (1.54 mg/l and 3.08 mg/l corresponding to .2 and .4 of the 48 hour LC_{50} dose, respectively) followed by an acutely lethal dose of 4-nitrophenol (1,000 mg/l). A significantly reduced survival time following exposure to 4-nitrophenol was reported. In a second experiment, snails were exposed to sublethal levels of 4-nitrophenol (13.2 mg/l) and subjected to a lethal temperature shock 96 hours later. A significant decrease in the median survival time of the snails during the temperature shock was noted. The applicability of these data to humans or mammals is unknown. Data regarding synergistic or antagonistic effects of the mononitrophenols in mammals were not found.

Teratogenicity

No information was found regarding the presence or absence of teratogenic properties of the mononitrophenols.

Mutagenicity

Szybalski (1958) tested the three mononitrophenol isomers for their ability to induce streptomycin-independence in streptomycin-dependent E. coli. All three isomers gave negative results.

Buselmaier, et al. (1976) tested 4-nitrophenol for mutagenic activity in mice with the host mediated assay and the dominant lethal method, using Salmonella typhimurium G46 HIS$^-$, Serratia marcescens a21, leu$^-$ and Serratia marcescens a31 HIS$^-$ as indicator organisms. Spot tests in vitro were also carried out. Mutagenic activity was not demonstrated.

4-nitrophenol also failed to induce mutations in Salmonella both with and without microsomal activation (McCann, et al. 1975).

Fahrig (1974) demonstrated a weak mutagenic activity when 4-nitrophenol was tested for mitotic gene conversion in Saccharomyces cerevisiae. This test-system allows the detection of a genetic alteration whose molecular mechanism is presumably based on the formation of single-strand breaks of DNA.

Adler, et al. (1976) used the difference in growth inhibition of wild type Proteus mirabilis and the corresponding repair-deficient strain as an indication of DNA damage. 4-nitrophenol showed some evidence of DNA damage in this system.

Effects on mitosis and chromosome fragmentation have been reported in plants. Sharma and Ghosh (1965) examined the mitotic effects of the mononitrophenol isomers in root tips of Allium cepa. Inhibition of mitosis in root tips was reported for all three mononitrophenol isomers but only 4-nitrophenol induced detectable chromosome fragmentations. Amer and Ali (1969) studied the effects of 2-nitro and 4-nitrophenol on the lateral root mitoses of Vicia faba seedlings. The mitotic index was reduced at concentrations of these compounds ranging from 0.025 percent to 0.1 percent. Induction of anaphase bridges by both isomers was noted but (in agreement with the work of Levin and Tjio (1948) with Allium cepa) chromosome fragmentation was not detected. The relationship of these changes in plants to alterations in mammalian cells has not been established. Based on the available data the mononitrophenols do not appear to pose a mutagenic hazard to humans.

Carcinogenicity

Data on the possible carcinogenicity of the mononitrophenols are scant in the literature. Boutwell and Bosch (1959) have studied the ability of a number of phenolic compounds to promote tumor formation on mouse skin following a single initiating dose of dimethylbenzanthracene. Although phenol itself has demonstrated a promoting capacity in this system both 2- and 4-nitrophenol failed to promote tumor development in mice. No other data on possible carcinogenic potential of the mononitrophenols were found.

4-nitrophenol has been selected by the National Cancer Institute for testing under the Carcinogenesis Bioassay Program.

DINITROPHENOLS

Mammalian Toxicology and Human Health Effects

EXPOSURE

Introduction

Six isomeric forms of dinitrophenol are possible, distinguished by the position of the nitro groups on the phenolic ring. Of the six possible dinitrophenol isomers, 2, 4-dinitrophenol is by far the most important. The most recent production figure for 2,4-dinitrophenol is 863,000 lb reported by the U.S. International Trade Commission (1968). Approximate consumption per year is estimated at 1,000,000 lb (Howard, et al, 1976). 2, 4-dinitrophenol is used primarily as a chemical intermediate for the production of sulfur dyes, azo dyes, photochemicals, pest control agents, wood preservatives, and explosives (Matsuguma, 1967; Perkins, 1919; Springer, et al. 1977a,b).

Production figures and usage data for the remaining five dinitrophenol isomers are not available. It is reasonable to assume that production and usage of these compounds are extremely limited in the United States.

Commercial synthesis of 2,4-dinitrophenol is accomplished by the hydrolysis of 2,4-dinitro-1-chlorobenzene with sodium hydroxide at 95 to 100°C (Matsuguma, 1967). As a result of the use pattern of 2,4-dinitrophenol (2,4-DNP) the major source for environmental release of 2,4-DNP is likely from production plants and chemical firms where the compound is used as an intermediate. It is possible that 2,4-DNP may also be produced via microbial or photodegradation of compounds which contain the dinitrophenol moiety, such as Parathion (Gomaa and Faust, 1972). 2,4-DNP has also been identified as an impurity in technical preparations of the herbicide DNPP (2-isopropyl-4,6-dinitrophenol) by Mosinska and Kotarski (1972).

The physical and chemical properties of the dinitrophenol isomers are summarized in Table 5.

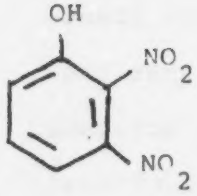
TABLE 5

Properties of Dinitrophenol Isomers[a]

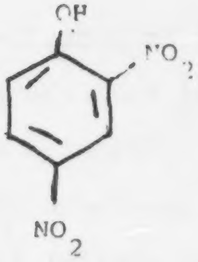
Isomer	m.p. (°C)	K (at 25°C)	Water Solubility (g/l)	Density
2,3-Dinitrophenol	144	1.3×10^{-5}	2.2	1.681
2,4-Dinitrophenol	114-115 (sublimes)	1.0×10^{-4}	0.79	1.683
2,5-Dinitrophenol	104	7×10^{-6}	0.68	
2,6-Dinitrophenol	63.5	2.7×10^{-4}	0.42	
3,4-Dinitrophenol	134	4.3×10^{-5}	2.3	1.672
3,5-Dinitrophenol	122-123	2.1×10^{-4}	1.6	1.702

[a] Source: Harvey, 1959; Windholz, 1976; Weast, 1975.

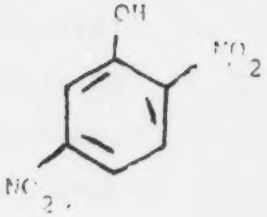
Dinitrophenols



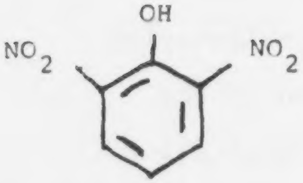
2,3-dinitrophenol



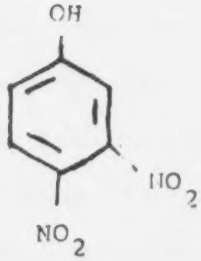
2,4-dinitrophenol



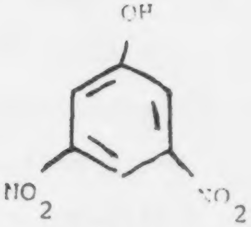
2,5-dinitrophenol



2,6-dinitrophenol



3,4-dinitrophenol



3,5-dinitrophenol

Ingestion from Water

No data were available regarding human exposure via ingestion of dinitrophenols from water.

The enhancement of biological waste water treatment by 2,4-DNP has recently been examined (Shah, 1975; Shah, et al. 1975). Addition of 0.92 mg/l 2, 4-DNP to waste water systems results in an increase of 85 percent in waste degrading rate and a decrease of 70 percent in cell growth. Shah, et al. (1975) note that the optimum concentration for 2,4-DNP in this system (0.92 mg/l) is undesirably high from the standpoint of current Federal effluent regulations but that the compound is completely eliminated by absorption on activated carbon which generally follows biological treatment of waste waters. It is not known whether this treatment method is currently used in the United States. Theoretically such usage might result in 2,4-DNP contamination of surface waters.

Games and Hites (1977) detected dinitrophenol (isomer not identified) in the effluent waters of a dye manufacturing plant. 400 to 3200 µg/l dinitrophenol was detected in raw waste water, prior to biological treatment. The final plant effluent contained 42 to 270 µg/l of dinitrophenol. Mud and river water samples downstream from the effluent point were analyzed by gas chromatography/mass spectromometry. Dinitrophenol was not detected in these samples.

The persistence of dinitrophenol isomers in ambient waters has not been well studied. A number of investigators have studied the bacterial degradation of the dinitrophenols utilizing acclimated populations of microorganisms. Phenol-adapted bacteria obtained from garden soil, compost, and river mud degraded 2,4-dinitrophenol in seven to ten days (Tabak, et al. 1964). 2,6-dinitrophenol was degraded very slowly in this system. 2,4-, 2,5-, and 2,6-dinitrophenol were tested for biological degradability by an activated sludge culture obtained from a sewage treatment plant (Pitter, 1976). 2,5-dinitro and 2,6-dinitrophenol were not degraded in this system although 85 percent removal of 2,4-dinitrophenol was achieved within 20 days. Further degradation of 2,4-dinitrophenol did not occur in this system, however. Bacteria isolated from parathion-treated flooded soil (Sudhakar-Barik, et al. 1976) degraded 2,4-dinitrophenol after an exceptionally long lag period. Nitrite was produced only in trace amounts after 25 days. Even after 50 days, only eight percent nitrogen was accounted for as nitrite.

The available data indicate that dinitrophenols are susceptible to partial degradation by certain microorganisms. Of the dinitrophenol isomers, 2,4-DNP appears to be most easily degraded. It may be speculated that dinitrophenols will be subject to microbial attack in environmental situations where acclimated microbiological populations exist (e.g. sewage treatment plants). The persistence of dinitrophenols in the environment where acclimated microbial populations do not exist is speculative.

Ingestion from Foods

No data were found demonstrating the presence of dinitrophenols in food.

No measured steady-state bioconcentration factor (BCF) is available for any nitrophenol; however, an estimated value can be derived by using a log equation (Veith, et al., Manuscript) based upon the octanol-water partition coefficient. Thus, the weighted average BCF for 2,4-dinitrophenol and the edible portion of all aquatic organisms consumed by Americans is 2.4 (Table 2A).

Inhalation

Dinitrophenol isomers may be produced in the atmosphere through a photochemical reaction between benzene and nitrogen monoxide. Nojima, et al. (1975) irradiated a combination of benzene vapor and nitrogen monoxide for five hours with a xenon lamp and characterized the following resulting photochemical products: nitrobenzene, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2,6-dinitrophenol. The authors suggested that these nitrocompounds may be the cause of the characteristic symptoms of seriously stricken victims of photochemical smog in Japan, which include headache, breathing difficulties, vomiting, rise in body temperature and numbness in the extremities. In the absence of monitoring data it is impossible to estimate the extent of human exposure to dinitrophenols as a result of their photochemical production in the atmosphere.

Dermal

2,4-DNP is rapidly absorbed through the intact skin (Gleason, et al. 1969). Although no direct information on the other dinitrophenol isomers is available, it is reasonable to suppose that dermal absorption will readily occur with these compounds as well. Since 2,4-DNP is used primarily as a chemical intermediate, dermal exposure is expected to occur most often in an industrial setting. 2, 4-DNP is also used occasionally as a spray against aphids and mites, as a fungicide for certain molds and mildews, as a weed killer, and as an ingredient in some wood preservative formulations (Gleason, et al. 1969). Dermal exposure to humans may occur among individuals handling 2,4-DNP in these applications. Direct data on the importance of the dermal exposure route of dinitrophenols in humans are not available.

PHARMACOKINETICS

Absorption

The dinitrophenol isomers are readily absorbed from the gastrointestinal tract based on the toxicological data to be presented in a later section. In addition, absorption through the skin and following inhalation readily occurs (von Oettingen, 1949).

Gehring and Buerge (1969b) reported that 2,4-DNP is absorbed extremely rapidly by ducklings and rabbits following intraperitoneal administration. In fact, immature rabbits absorbed the administered DNP so rapidly that an absorption constant could not be calculated from the data. DNP concentration in serum peaked within five minutes of administration

Other quantitative information on the rate of absorption of the dinitrophenol isomers was not found.

Distribution

Blood levels of the dinitrophenols rise rapidly following absorption (Gehring and Buerge, 1969b; Harvey, 1959) suggesting that the dinitrophenol isomers are transported by the blood regardless of the mode of absorption. 2,4-DNP binds to serum proteins following intraperitoneal administration to rabbits and ducklings. Early after the administration of 2,4-DNP the concentration of free DNP in serum is much greater than the bound form, and at later times the reverse is true (Gehring and Buerge, 1969b).

Based on the available data, the dinitrophenol isomers are not stored to any significant extent in the tissues of human or experimental animals following absorption. Gisclard and Woodward (1946) unsuccessfully attempted to extract 2, 4-dinitrophenol or its metabolites from the tissues of two human victims of fatal intoxication.

It seems likely, based on the short half-lives of these compounds in mammals, that the large majority of any dose will be rapidly excreted via the urine. On the other hand, von Oettingen (1949) reported both dinitrophenol (unspecified isomer) and amino nitrophenol in liver, kidney, brain, blood, and spinal fluid of dogs after fatal doses of dinitrophenol. Recent work on the tissue distribution of the dinitrophenols following absorption in mammals was not found.

Metabolism

In a study of the munitions industry in France (Perkins, 1919) it was reported that the urine of men fatally poisoned by 2,4-DNP contained: amino-2-nitro-4-phenol, amino-4-nitro-2-phenol, diamino-phenol, and a number of nitrogen compounds resulting from a combination of two molecules of amino-nitrophenol or of diamino-phenol. It has frequently been reported that 2-amino-4-nitrophenol invariably exists in the urine of persons suffering from serious intoxication by

2,4-DNP. Williams (1959) stated that 2,4-DNP is excreted in mammals in the following forms: partially unchanged; partially conjugated with glucuronic acid; reduced to 2-amino-4-nitrophenol, 2-nitro-4-aminophenol and probably 2,4-diaminophenol. Rats orally dosed with 1.5 to 12 mg/kg of 2,4-DNP excreted both free dinitrophenol (78 percent) and 2-amino-4-nitrophenol (17 percent) (Senszuk, et al. 1971).

Although the in vitro metabolism of 2,4-dinitrophenol has not been extensively studied in mammalian systems, Parker (1952) examined the enzymatic reduction of 2,4-DNP by rat liver homogenates and found 4-amino-2-nitrophenol to be the major metabolite. The metabolite 2-amino-4-nitrophenol comprised less than 10 percent of the total metabolites formed; 2,4-diaminophenol was found in trace amounts. Presumably the latter metabolite was formed from the reduction of the remaining nitro group of one of the two above compounds.

In contrast, Eiseman, et al. (1974) reported 2-amino-4-nitrophenol was the major metabolite (75 percent of total amine). In the latter report 4-amino-2-nitrophenol was found to have been formed in considerably lesser amounts (23 percent) when 2,4-DNP was enzymatically reduced in vitro by rat liver homogenates. These investigators also detected only traces of diaminophenol indicating that it may be a secondary reduction product as suggested by Parker (1952). A precise definition of the metabolic fate of the dinitrophenols in humans awaits further investigation.

Excretion

Data on the elimination kinetics of the dinitrophenols or their metabolic products in humans were not found. Edsall (1934) stated "judging from the metabolic response, DNP appears to be eliminated entirely in three or four days; in the presence of liver or kidney damage it is possible that the drug will be retained over a longer period." Information on the elimination kinetics of the dinitrophenols from experimental animals is also scant in the literature.

Gehring and Buerge (1969b) have developed equations which describe the elimination of 2,4-DNP from the serum of ducklings, mature rabbits, and immature rabbits following intraperitoneal administration of the compound. Serum levels of 2,4-DNP in the mature rabbit declined to less than one percent of their original high values within seven hours. Twenty- four hours were required before the serum levels in the immature rabbit declined to two percent of their original values. Ducklings eliminated 2,4-DNP from the serum over a similar time frame (96 percent elimination in 24 hours).

Lawford, et al. (1954) also studied the elimination of various nitrophenolic compounds (including 2,4-dinitrophenol). Elimination from the blood of mice, rabbits, guinea pigs, rats, and monkeys was complete within 30 hours. Harvey (1959) calculated the elimination rates of all six dinitrophenol isomers from the blood of mice and rats following a single large dose given intraperitonealy. His data are presented in Table 6. The data developed by these investigators

TABLE 6

Elimination Rates of Dinitrophenol Isomers from the Blood of Mice and Rats Following a Single Large Intraperitoneal Dose

Isomer	Dose (mg/kg)	Half-time for Elimination (min.)
	MICE	
2,3-Dinitrophenol	90	2.7
2,4-Dinitrophenol	20	54.0
2,5-Dinitrophenol	180	3.3
2,6-Dinitrophenol	30	238.0
3,4-Dinitrophenol	60	3.5
3,5-Dinitrophenol	30	2.7
	RATS	
2,3-Dinitrophenol	90	12.5
2,4-Dinitrophenol	20	225.0
2,5-Dinitrophenol	90	13.0
2,6-Dinitrophenol	25	210.0
3,4-Dinitrophenol	90	11.5
3,5-Dinitrophenol	30	2.1

Source: Modified from Harvey, 1959.

must be taken with caution since the actual elimination of the dinitrophenols or their metabolites in urine was not directly measured. In view of the lack of data suggesting concentration of the dinitrophenols in mammalian tissues and their high water solubility, it is suggested that their elimination via the urine may be a rapid process in humans.

EFFECTS

Acute, Sub-acute, and Chronic Toxicity

All of the dinitrophenol isomers are potent metabolic poisons. Most of the literature available deals with 2,4-dinitrophenol since this compound has been used extensively for more than 70 years. A number of excellent reviews on the uses, chemistry, mode of action, and mammalian toxicity of 2,4-dinitrophenol are available (Edsall, 1934; Metcalf, 1955; Horner, 1942; Simon, 1953; Slater, 1962; Parascandola, 1974; Howard, et al. 1976) and no attempt will be made to duplicate the information found in these documents.

2,4-dinitrophenol is considered a classic uncoupler of oxidative phosphorylation and is widely used by biochemists to determine whether a given biochemical process is energy dependent. Hence, an enormous body of literature has been generated dealing with the biochemical effects of 2,4-dinitrophenol on cellular and biochemical processes both *in vivo* and *in vitro*. Only those studies with direct relevance to the acute or chronic effects of the dinitrophenols on humans are reviewed in this document.

The toxic action of the dinitrophenols is generally attributed to their ability to uncouple oxidative phosphorylation. These compounds prevent the utilization of the energy provided by respiration and glycolysis by inhibiting the formation of high energy phosphate bonds. All energy dependent biochemical processes are therefore affected by the action of the compounds (Metcalf, 1955). The large number of clinical effects attributed to dinitrophenol toxicity result essentially from the short-circuiting of metabolism in cells which absorb sufficient dinitrophenol.

All six dinitrophenol isomers are potent uncouplers of oxidative phosphorylation. The relative potencies of the six dinitrophenols in uncoupling phosphorylation in rat liver mitochondria were found to be (in declining order): 3,5-> 2,4-> 2,6- = 3,4-> 2,3- = 2,5-dinitrophenol (Burke and Whitehouse, 1967). 3,5-dinitrophenol is approximately five times more potent than 2,5-dinitrophenol as measured in this system. The relative *in vivo* toxicities of the dinitrophenol isomers have been determined by a number of investigators (von Oettingen, 1949; Harvey, 1959; Cameron, 1958; Grant, 1959; Levine, 1977) and the order of relative potency of the isomers determined in these investigations frequently differs from the order developed by Burke and Whitehouse (1967). Several explanations for these discrepancies are possible: (1) differential tissue absorption of the isomers, (2) different metabolic detoxification mechanisms for the isomers or (3) the presence of cellular or biochemical effects unrelated to the uncoupling of oxidative phosphorylation. Resolution of this question awaits further investigation.

At concentrations higher than those necessary to uncouple oxidative phosphorylation, a number of inhibitory effects of the dinitrophenol isomers on certain enzymatic reactions may occur.

Both 2,4-dinitro and 3,5-dinitrophenol inhibit porcine heart malate dehydrogenase in vitro (Wedding, et al. 1967). Inhibition of the reaction occurred at nitrophenol concentrations 10 to 100 times those causing uncoupling, and resulted from a competitive inhibition with NAD in the forward direction of the malate dehydrogenase reaction. In a similar study Stockdale and Selwyn (1971) reported the in vitro inhibition of both lactate dehydrogenase and hexokinase by 2,4-dinitro, 2,5-dinitro, and 2,6-dinitrophenol.

The dinitrophenols may also act directly on the cell membrane, thus causing toxic effects on cells which do not depend on oxidative phosphorylation for their energy requirements. 2,4-dinitro, 2,5-dinitro, and 2,6-dinitrophenol inhibit passive chloride permeability (a metabolic independent process) in red blood cells (Motais, et al. 1978).

Acute toxicity information for the dinitrophenols has been compiled and presented in Table 7.

Numerous occasions of human poisoning by 2,4-DNP have been reported in the literature. The earliest cases of fatal 2,4-DNP intoxication relate to its usage as a component of explosives during World War I. Thirty-six cases of fatal

TABLE 7

Acute Toxicity of Dinitrophenol Isomers

Species	Dose (mg/kg)	Route of Adminisration	Effects	References
		2,4-Dinitrophenol		
Rat	25	S.C.	LD 50	von Oettingen, 1949
Rat	35	I.P.	LD 50	Harvey, 1959
Rat	30	Oral	LD 50	Spector, 1956
Rat	28.5	I.P.	LD 50	Lawford, et al. 1954
Rat	31	I.P.	LD 100	Gatz and Jones, 1970
Mouse	36	I.P.	LD 50	Harvey, 1959
Mouse	26	I.P.	LD 50	Lawford, et al. 1954
Guinea Pig	700	Dermal	Lethal Dose	Spencer, et al. 1948
Rabbit	30	S.C.	LD 50	von Oettingen, 1949
Rabbit	200	Oral	LD 50	Spector, 1956
Rabbit	100	I.P.	Lethal Dose	Spector, 1956
Dog	30	UNK	Minimum Lethal Dose	Harvey, 1959
Dog	20-30	Oral	LD 50	Spector, 1956
Dog	22	S.C.	LD 50	Spector, 1956
Dog	20	I.M.	LD 50	Spector, 1956

TABLE 7 (Continued)

Species	Dose (mg/kg)	Route of Administration	Effects	References
Dog	30	I.V.	LD 50	Spector, 1956
Pigeon	7	I.M.	LethaL Dose	Spector, 1956
Pigeon	15-20	I.V.	Lethal Dose	Spector, 1956
Human	40 mg/m^3	Inhalation	Lethal Concentration	MacBryde and Taussig, 193
Human	1-3 g	Oral	Lethal Dose	Sax, 1968
Human	4.3	Oral	Lethal Dose	Geiger, 1933
		2,3-Dinitrophenol		
Rat	190	I.P.	LD 50	Harvey, 1959
Mouse	200	I.P.	LD 50	Harvey, 1959
Dog	1000	UNK	MLD	Harvey, 1959
		2,5-Dinitrophenol		
Rat	150	I.P.	LD 50	Harvey, 1959
Mouse	273	I.P.	LD 50	Harvey, 1959
Dog	100	UNK	MLD	Harvey, 1959

TABLE 7 (Continued)

Species	Dose (mg/kg)	Route of Administration	Effects	References
		2,6-Dinitrophenol		
Rat	38	I.P.	LD 50	Harvey, 1959
Mouse	45	I.P.	LD 50	Harvey, 1959
Dog	50	UNK	MLD	Harvey, 1959
		3,4-Dinitrophenol		
Rat	98	I.P.	LD 50	Harvey, 1959
Mouse	112	I.P.	LD 50	Harvey, 1959
Dog	500	UNK	MLD	Harvey, 1959
		3,5-Dinitrophenol		
Rat	45	I.P.	LD 50	Harvey, 1959
Mouse	50	I.P.	LD 50	Harvey, 1959
Dog	500	UNK	MLD	Harvey, 1959

occupational dinitrophenol poisoning occurred among employees of the munitions industry in France between 1916 and 1918 (Perkins, 1919). A literature review by von Oettingen (1949) revealed 27 reported cases of fatal occupational dinitrophenol poisoning in the United States for the years 1914 to 1916.

Gisclard and Woodward (1946) reported two fatal cases of dinitrophenol poisoning during manufacture of picric acid where 2,4-DNP was produced as an intermediate. Swamy (1953) describes a case of suicidal poisoning by 2,4-DNP.

Early in the 1930's, 2,4-dinitrophenol was widely recommended as a treatment for obesity. Dinitrophenol was received with overwhelinmg popularity (Horner, 1942) as a slimming agent in spite of warnings of harmful side effects caused by disruption of the metabolic rate. It was estimated that during the first 15 months following its introduction, 100,000 persons took the drug for weight reduction (Horner, 1942). More than 1,200,000 capsules of 0.1 g each were dispensed from a single clinic in San Francisco. More than 20 drug houses offered to supply both dinitrophenol and mixtures containing the drug. Many of these remedies could be procured without prescription and with no further directions than to take "one capsule three times daily after meals." In view of this widespread and uncontrolled usage of the compound it is not surprising that both toxic side effects and fatalities resulted. Horner (1942) reported a total of nine deaths resulting from the use of dinitrophenol as a slimming agent.

Parascandola (1974) reviewed the history and public concern which developed over dinitrophenol in the United States. An article appearing in Newsweek (1933) entitled "Diet and Die with Excess Alpha Dinitrophenol" was typical of public concern generated by misuse of dinitrophenol. In the wake of reports that cataract development in humans attributable to dinitrophenol was occurring, the drug was finally withdrawn from use in 1937.

The toxic manifestations of dinitrophenol exposure as reviewed by Horner (1942), included subacute symptoms such as gastrointestinal disturbances (nausea, vomiting, colic, diarrhea, anorexia) profuse sweating, weakness, dizziness, headache, and loss of weight. Acute poisoning has resulted in the sudden onset of pallor, burning thirst, agitation, dyspnea, profuse sweating, and hyperpyrexia. Intense and rapid onset of rigor mortis after death has also been described. A physician who ingested a fatal overdose of dinitrophenol (estimated at 2.5 to 5 g) was literally "cooked to death" (Geiger, 1933). Rectal temperature at death exceeded 110°F.

Perkins (1919) made the interesting observation that post-mortem examination of dinitrophenol victims demonstrated no characteristic lesions. Acute edema of the lungs was mentioned but was believed to be secondary to toxic effects on the vasomotor system. Microscopic lesions of the liver and kidney cells were inconstant and typical changes were lacking elsewhere.

Spencer, et al. (1948) studied the chronic toxicity of 2,4-dinitrophenol in rats. Male rats were fed diets containing 0.01, 0.02, 0.05, 0.10, or 0.20 g of 2,4-dinitrophenol per 100 g of food. Rats were maintained on diets containing 2,4-dinitrophenol for six months and both hematological and pathological investigations on surviving animals were carried out. Hematological examination included erythrocyte counts, hemoglobin concentrations, leukocyte counts, differential counts, and bone marrow counts at autopsy. Both gross and microscopic examination of liver, kidney, spleen, lung, heart, adrenal, pancreas, and stomach tissues were also carried out. Rats maintained on diets containing .02 percent 2,4-DNP (corresponding to 5.4 to 20 mg/kg body weight/day) grew at a normal rate and the investigators failed to detect discernible ill effects or pathological changes at autopsy. Similary pathological changes were not found upon microscopic examination of tissues from rats receiving diets containing .05 percent 2,4-DNP (corresponding to 13.5 to 50 mg/kg/day) although growth of these rats fell five to ten percent below that of the controls throughout the six-month experimental period. At autopsy the only changes observed in these animals were a very slight depletion of body fat and a very slight increase in the average weight of the kidneys. At higher doses of 2,4-dinitrophenol in their diets (54 to 200 mg/kg body weight/day) rats occasionally died and survivors lost weight rapidly. Examination of surviving animals revealed marked emaciation, an empty gastrointestinal tract, a slightly enlarged dark spleen, and small testes. Microscopic examination showed slight congestion and cloudy swelling of the liver, very slight parenchymatous degeneration of the epithelium of the renal tubules, slight congestion and hemosiderosis of the spleen, and testicular atrophy. No significant pathological changes were observed in the lung, heart, adrenals, pancreas, or stomach of these animals. Based on the work of Spencer, et al. (1948), the no observable effect level for 2,4-DNP in rats lies between 5.4 and 20 mg/kg body weight/day.

Information on the subacute or chronic effects of the other dinitrophenol isomers in experimental animals was not found. Langerspetz and Tarkkonen (1961) failed to detect histological changes in the adrenals or the liver during 2,4-dinitrophenol treatment of Swiss albino male mice. 2,4-dinitrophenol was administered via the subcutaneous injection of 10 mg, 2,4-DNP/kg twice daily for 30 days.

Arnold, et al (1976) examined the effects on the kidney of a single large dose of 2,4-DNP. Although a dose close to the LD 50 was chosen (20 mg/kg) only small areas of cortical tubular necrosis were observed in a few of the rats treated.

Tainter and Cutting (1933) administered 2,4-DNP to dogs at intervals of three or more days over a period of two to three months. Liver and kidney pathology were not detected but an effect on spleen tissue was noted. Over large areas of the spleen lymphocytes were replaced by a more or less homogenous material containing "numerous large faintly staining cells with vesicular polyhedral nuclei."

The widespread use of 2,4-dinitrophenol as a weight reducing agent in humans during the 1930's provides some information regarding the chronic effects of this compound in man. Recommended theraputic doses of 2,4-DNP for weight control in humans ranged from 2 to 5 mg/kg body weight/day (Dunlop, 1934; Horner, 1942; Tainter, et al. 1933). Tainter, et al. (1933) administered 2,4-DNP to 113 obese patients for as long as four months without demonstrating evidences of cumulative or toxic effects. The most important side effect noted by the investigator was a skin rash observed in about seven percent of the patients treated. The rash was manifested usually after a one-day period of mild itching and consisted of a maculopapular or urticarial type of rash. The itching was intense and in some cases there was considerable swelling. Symptoms subsided in two to five days following withdrawal from the drug. The next most important side effect noted by the authors was a loss of taste for salt and sweets observed in 5.3 percent of the patients. This effect also cleared up following withdrawal from 2,4-DNP. The investigators failed to detect any effect of 2,4-DNP on liver or kidney function, pulse, blood pressure, or oxygen capacity of the blood. No cases of anemia, agranulocytosis, or malignant neutropenia appeared. Three cases of mild gastrointestinal upset were reported, however.

The development of cataracts following dinitrophenol therapy was first described by Horner, et al. (1935). In a later publication, Horner (1942) reviewed the acute and chronic toxicity of 2,4-DNP (including cataract formation) resulting from therapeutic use of the compound. Gastrointestinal symptoms consisting of nausea, vomiting, and loss of appetite were common as a result of 2,4-DNP administration. Cutaneous lesions were the most frequent side effect with an incidence of 8 to 23 percent. Although the majority of lesions were mild, others were severe. Bone marrow effects of dinitrophenol have also been reported. Eight cases of agranulocytosis were reported with three fatalities. Thirty cases of neuritis including aberations of taste and multiple regional involvement affecting, particularly, the feet and legs were recorded. Symptoms appeared after an average of ten weeks, followed ordinary therapeutic doses and persisted for weeks or months. Electrocardiographic evidence of functional heart damage was offered by several investigators and fragmentation of the heart muscle at autopsy in one fatal case was reported. It was generaly agreed that 2,4-DNP was rarely injurious to the liver and kidneys when administered in therapeutic doses.

Over 100 cases of cataract formation following dinitrophenol therapy were reviewed by Horner (1942). Horner described the following characteristic features of 2,4-DNP induced cataracts: 1. They occurred in young women who were physically normal save for varying degrees of obesity and were in an age group in which senile cataracts do not occur. 2. They were bilateral and appeared either during or after periods of dinitrophenol treatment. 3. An interval of months or years might elapse between the time the last dose was taken and the onset of blurred vision. 4. Lenticular changes were strikingly similar and could be demonstrated with the biomicroscope at a time when vision for distance and reading was still normal. 5. After gradual onset, the lenticular changes progressed with startling rapidity until the vision was obscured. 6. Treatment was without effect in staying their progress. 7. Surgical removal of the lens was uniformly successful in restoring vision.

The length of time that 2,4-DNP was taken and the amount of the drug consumed varied widely among cataract victims. In 29 cases the duration of treatment varied from 3 months to 24 months with an average of 11 months. Neither the length of treatment nor the total dose seemed to have any bearing on the occurrence of cataracts. Individual susceptibility appeared to be a more important factor. Horner (1942) estimated that the incidence of cataracts in patients who had taken dinitrophenol exceeded one percent.

Formation of cataracts by acute exposure to DNP was first demonstrated in animals almost ten years after the problem was known to exist in humans (Gehring and Buerge, 1969a; Ogino and Yasukura, 1957; Feldman, et al. 1959, 1960; Bettman, 1946). Experimental cataracts, first produced in ducks and chickens, differ from DNP-induced human cataracts in that they can be formed in acute exposures and may appear in less than one hour. Furthermore, these lesions will disappear spontaneously in animals within 25 hours (Howard, et al. 1976). Hence, the usefulness of data on the formation of cataracts in experimental animals following DNP administration to assessing human hazard to dinitrophenol is questionable.

The available data do not allow the calculation of a minimum effect level for 2,4-DNP induced cataract formation in man. Cataractogenic activity in humans has been observed in a small proportion of patients receiving as little as 2 mg/kg body weight/day. An assessment of the no-effect level for cataract formation awaits further investigation. Such an assessment is further complicated by the fact that cataract formation in humans, following DNP administration, differs significantly from the situation seen in experimental animal studies.

Synergism and/or Antagonism

A report of teratogenic synergism following the combined administration of 2,4-dinitrophenol and insulin to chicks was made by Landauer and Clark (1964). The injection of 100 µg/egg of 2,4-dinitrophenol was nontoxic and nonteratogenic after 96 hours of incubation. However, the combined administration of insulin (a known teratogen) with 100 micrograms of 2,4-dinitrophenol raised the incidence of embryo mortality from 16 to 19 percent and shortened the upper beak by 1.4 to 18.5 percent.

Both thyroid hormones and 2,4-dinitrophenol decrease the efficiency of mitochondrial oxidative phosphorylation in vivo and in vitro. The in vivo administration of both L-thyroxine and 2,4-DNP results in larger changes in metabolic rate and body temperature than are accounted for by the sum of the separate effects of each agent (Hoch, 1965).

Other direct information on possible synergism between the dinitrophenols and other chemical compounds is not available.

Teratogenicity

Wulff, et al (1935) examined the effects of 2,4-dinitrophenol on the fertility, gestation, and fetal life of rats. They administered 20 mg of 2,4-DNP/kg to female rats eight days prior to the introduction of males. Dinitrophenol was administered intragastrically twice daily until the respective litters were weaned. The average number born in each litter was not affected by the use of dinitrophenol. Neither did the treatment appreciably affect the body weight gains of mothers during pregnancy. Neonatal malformations were not detected. Among 2,4-dinitrophenol treated rats, however, 25 percent of the total number of young were stillborn while only 6.8 percent of the young were stillborn in the control group. In addition, the mortality during the nursing period of viable young born to 2,4-DNP mothers was 30.9 percent as compared with 13.4 percent for young of control mothers. Two possible explanations for this latter phenomenon were offered: Dinitrophenol mothers neglect their young while in a febrile state, and only the more vigorous of the offspring manage to reach the mother for nursing; or, a toxic agent is passed to the young through the milk. Data to distinguish between the two possibilities are not available.

Intraperitoneal (7.7 or 13.6 mg/kg) or oral (25.5 or 38.3 mg/kg) administration of 2,4-DNP to mice during early organogenesis does not produce morphological defects in the young, but embryotoxicity occurs at the higher dose levels (Gibson, 1973). The higher doses also produced overt toxic signs (hyperexcitability and hyperthermia) in the dams, but were not lethal.

Bowman (1967) has studied the effect of 2,4-DNP on the developing chick embryo *in vitro*. At 2,4-DNP concentrations of 18 mg/l or 370 mg/l a syndrome of abnormalities resulted, consisting of degeneration and sometimes complete absence of neural tissue accompanied by a reduction in the number of somites. The 2,4-DNP concentrations used in this study are extremely high and the relevance of the experimental findings to the *in vivo* situation in mammals is unknown.

Malformations such as hemiophthalmus and cross beak were induced in chick embryos following administration of 0.5 μm/egg (92 μg/egg) into the yolk sack at 48 hours of incubation (Miyamoto, et al. 1975). Based on examination of purified myelin in the malformed embryos the investigators suggested that 2,4-DNP administration resulted in deficient embryonic myelination.

Based on the available data it appears unlikely that the dinitrophenols pose a teratogenic hazard to humans. Further investigations on this question are warranted.

Mutagenicity

Friedman and Staub (1976) have developed an approach to mutagenic testing which utilizes the measurement of induction of unscheduled DNA synthesis in testes. These investigators found a good correlation between a reduction in the residual level of cell cycle associated DNA synthesis and the presence of known mutagenic compounds. Testicular DNA synthesis in mice was unaffected by administration of 2,4-DNP suggesting a lack of mutagenic activity.

Bacterial mutagenesis of 2,4-DNP has been tested by Demerec, et al. (1951), based on the production of back mutations from streptomycin dependence to independence in *E. coli*. Mutations were increased several-fold over control values.

A recent study has been conducted on the effect of various phenolic compounds including 2,4-DNP on chromosomes of bone marrow cells from mice (Mitra and Manna, 1971). Mice were injected intraperitoneally with 2,4-DNP and bone marrow tissue was collected 24 hours after treatment. The results suggest that 2,4-DNP may produce chromatid type breaks in bone marrow cells. However, there was no linear relationship between the frequency of chromosome aberrations and the dose of 2,4-DNP.

It is possible to make a rough estimate of the 2,4-DNP doses administered to the mice by these investigators. The water solubility of 2,4-DNP at 75.8°F is 3.01 mg/ml (Windholz, 1976). If this value approximates the saturated solution used by Mitra and Manna (1971) and a three-to-four month old mouse weighs approximately 40 g, the following calculations result in three 2,4-DNP dose levels expressed as mg/kg body weight.

$$\frac{(0.25\ \text{ml})\ (3.01\ \text{mg/ml})}{.04\ \text{kg}} = 18.8\ \text{mg/kg}$$

$$\frac{(0.5\ \text{ml})\ (3.01\ \text{mg/ml})}{.04\ \text{kg}} = 37.6\ \text{mg/kg}$$

$$\frac{(1.0\ \text{ml})\ (3.01\ \text{mg/ml})}{.04\ \text{kg}} = 75.3\ \text{mg/kg}$$

The ability of 2,4-DNP to induce chromosomal damage using an in vitro alkaline elution assay employing Chinese hamster V79 cells (with or without a liver microsomal activation DNP system) was examined by Swenberg, et al. 1976). 2, 4-DNP failed to induce DNA damage in this system.

Data addressing the possible mutagenicity of the other dinitrophenol isomers were not found.

Carcinogenicity

In a study designed to measure tumor promoting activity, Boutwell and Bosch, (1959) examined the ability of 2,4-DNP to promote tumor formation following a single initiating dose of dimethylbenzanthracine. Although phenol itself has a promoting activity in this system, 2,4-DNP failed to promote skin tumors in mice under similar conditions. In a similar experiment, Stenback and Garcia (1975) examined the ability of 2, 4-DNP to promote skin tumor formation in mice. No promoting activity was demonstrated.

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Spencer, et al. (1948) failed to detect tumor formation during chronic administration of 2,4-DNP to mice (over a six month period).

The available data suggest that 2,4-DNP does not possess carcinogenic properties. Information on the other isomeric dinitrophenols is not available.

TRINITROPHENOLS

Mammalian Toxicology and Human Health Effects

EXPOSURE

Introduction

Six isomeric forms of trinitrophenol are possible, distinguished by the position of the nitro groups relative to the hydroxy group on the six carbon benzene ring. The five isomers are: 2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6- and 3,4,5-trinitrophenol. Production volumes for the trinitrophenols are not available. Usage of the trinitrophenol isomers is apparently limited to 2,4,6-trinitrophenol, otherwise known as picric acid. In fact, a comprehensive search of the literature failed to detect a single citation dealing with any of the trinitrophenol isomers except picric acid. Consequently, the only information on these isomers presented in this document are the chemical and physical properties found in Table 8.

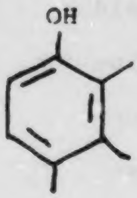
According to Matsuguma (1967) picric acid has found usage as: a dye intermediate, explosive, analytical reagent, germicide, fungicide, staining agent and tissue fixative, tanning agent, photochemical, pharmaceutical, and a process material for the oxidation and etching of iron, steel and copper surfaces. The extent to which picric acid finds usage in any of these applications at the present time is unknown.

TABLE 8

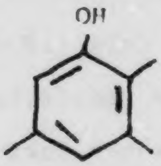
Properties of Trinitrophenols

2,3,4-Trinitrophenol	
Molecular Weight	229.11
2,3,5-Trinitrophenol	
Molecular Weight	229.11
Melting Point	119-120°C
2,3,6-Trinitrophenol	
Molecular Weight	229.11
Melting Point	119°C
Water Solubility	
Room Temperature	Slightly Soluble
Hot Water	Very Soluble
2,4,5-Trinitrophenol	
Molecular Weight	229.11
Melting Point	96°C
Water Solubility	
Room Temperature	Slightly Soluble
Hot Water	Soluble
2,4,6-Trinitrophenol	
Molecular Weight	229.11
Melting Point	122-123°C
Boiling Point	Sublimates: Explodes at 300°C
Vapor Pressure	1 mm Hg at 195°C
Density	1.763 g/cm^3
Water Solubility	
Room Temperature	1.28 g/l
100°C	6.7 g/l

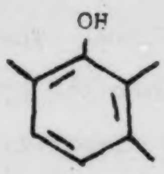
Trinitrophenols



2,3,4-trinitrophenol



2,3,5-trinitrophenol



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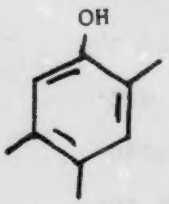

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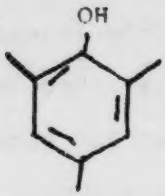
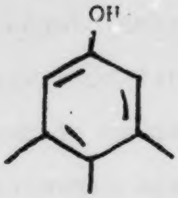


2,4,6-trinitrophenol



3,4,5-trinitrophenol

Ingestion from Water

Monitoring data on the presence or absence of 2,4,6-trinitrophenol (2,4,6-TNP) in water were not found. A single report of 2,4,6-TNP contamination of ground water was found, however (Cole, 1974). In 1955, 2,4,6-TNP (0.7 mg/l) was detected in a well approximatley one mile from the former site of an explosives manufacturing plant in England. The plant was engaged in the manufacture of explosives from 1914 to 1918. The brief report by Cole (1974) failed to describe either the types of explosives manufactured by the plant or the disposition of the waste water during the period of explosives manufacture.

Harris, et al. (1946) described an outbreak of hematuria involving U.S. Navy personnel aboard ships anchored at Wakayama, Japan which resulted from ingestion of 2,4,6-TNP in the drinking water. Approximatley three weeks prior to the outbreak of hematuria, more than 100 tons of confiscated Japanese ammunition, (including 2,4,6-TNP) had been dumped in the immediate vicinity of the anchorage. 2,4,6-TNP was apparently pumped into the ships' drinking water stills and carried over with the vapor phase and into the freshwater supply, inducing hematuria among those drinking the water. The investigators failed to detect 2,4,6-TNP in the sea water; however, analysis of the distilled drinking water yielded 2,4,6-TNP levels of 2 to 20 mg/l.

Although it is not possible to precisely estimate either the TNP water levels or duration of exposure necessary to induce hematuria, Harris, et al. (1946) detected levels of 10 mg/l and 20 mg/l in drinking water aboard two ships at the time of the hematuria outbreak.

Hoffsommer and Rosen (1973) have shown that the high explosive tetryl (N-methyl-N-nitro-2,4,6-trinitroaniline) dissolved in sea water at pH 8.1 and 25°C is largely converted to 2,4,6-TNP in a few months. Although tetryl is no longer manufactured in the U.S. (Howard, et al. 1976), these experiments indicate that 2,4,6-TNP may be produced in water as a result of degradation of other organic compounds. The nature of other compounds which may give rise to 2,4, 6-TNP following degradation is speculative.

The persistence of 2,4,6-TNP following release to the environment is not well understood. Pitter (1976) failed to detect degradation of 2,4,6-TNP using an acclimated activated sludge system with 2,4,6-TNP as a sole source of carbon for the microbes in the inoculum. Tabak, et al. (1964) on the other hand were able to demonstrate 95 percent degradation of 2,4,6-TNP (250 mg/l) by acclimated cultures of microorganisms derived from garden soils, compost, and river mud in three to six days. The extent to which microbial populations capable of degrading 2,4,6-TNP exist in the environment is unknown.

No other data on possible ingestion of 2,4,6-TNP from water by humans were found.

Ingestion from Foods

No information on human ingestion of trinitrophenols from food was found.

No measured steady-state bioconcentration factor (BCF) is available for any nitrophenol; however, an estimated value can be derived by using a log equation (Veith, et al., Manuscript) based upon the octanol-water partition coefficient. Thus, the weighted average BCF for 2,4,6-trinitrophenol and the edible portion of all aquatic organisms consumed by Americans is 6.0 (Table 2A).

Inhalation

No information on the presence or absence of trinitrophenols in air was found.

Dermal

Information on the dermal absorption of 2,4,6-TNP is scant in the literature. During the 1920's and 30's, 2,4,6-TNP was used both alone and in combination with butesin (dinormalbutyl-p-aminobenzoate trinitrophenol) as an antiseptic surgical dressing for the treatment of burns. Ehrenfried (1911) remarked on the dangers of poisoning by absorption of 2,4,6-TNP in dermal ointments, but added that, if the ointments were properly used, there was no danger of toxic symptoms developing in humans.

A serious case of central nervous system dysfunction following the topical application of 2,4,6-TNP was reported by Dennie, et al. (1929). The patient recovered rapidly following removal from the 2,4,6-TNP treatment. No other information on dermal absorption of the trinitrophenols by humans or experimental animals was found.

PHARMACOKINETICS

Absorption

Quantitative information on the absorption of 2,4,6-TNP by humans or experimental animals is not available.

Neurological complications following the topical administration of 2,4,6-TNP (Dennie, et al. 1929) indicate that the compound may be absorbed through the skin. Since the compound was applied to a burned area of the patient, the relevance of this data to the absorption of 2,4,6-TNP through intact skin is questionable.

The occurrence of human cases of microscopic hematuria resulting from ingestion of 2,4,6-TNP in drinking water (Harris, et al. 1946) and the known oral toxicity of 2,4,6-TNP in experimental animals indicate that absorption by the gastrointestinal tract readily occurs.

Distribution

Autopsy examination of dogs after a lethal dose of 2,4,6-TNP (Dennie, et al. 1929) revealed yellow staining of the subcutaneous fat, lungs, intestines, and the blood vessels. The results indicate that 2,4,6-TNP is distributed to many tissues in the body. These investigators also demonstrated the presence of 2,4,6-TNP in the blood and suggested that the compound may be bound to serum proteins. It seems likely the distribution of 2,4,6-TNP would occur via the blood. No other data on the tissue distribution of 2,4,6-TNP following absorption were found.

Metabolism

In a review of the early literature, Burrows and Dacre (1975) indicated that elimination of 2,4,6-TNP from humans occurs in both the free form and as picramic acid. In perfusion experiments with liver, kidney and spleen, the liver exhibited the strongest capacity for reduction of 2,4,6-TNP.

Other studies dealing with the metabolism of 2,4,6-TNP in humans or in experimental animals were not found.

Decompositon of 2,4,6-TNP by an atypical strain of Corynebacterium simplex with the production of nitrites has been reported by Gunderson and Jensen (1956). This alternative metabolic pathway for 2,4,6-TNP has not been reported in mammals.

Excretion

The presence of 2,4,6-TNP in blood and urine within 1.5 hours after administration of a lethal dose in dogs was reported by Dennie, et al. (1929). The presence of 2,4,6-TNP in the urine of humans following oral exposure was reported by Harris, et al. (1946). These studies indicate that 2,4,6-TNP is partially excreted in the urine following exposure. Other data on the excretion of 2,4,6-TNP were not found.

EFFECTS

Acute, Sub-acute, and Chronic Toxicity

According to Windholz (1976) ingestion or percutaneous absorption of 2,4,6-TNP may cause nausea, vomiting, diarrhea, abdominal pain, oliguria, anurea, yellow staining of skin, pruritus, skin eruptions, stupor, convulsions, and death.

Although Dennie, et al. (1929) state: "The application of a solution of trinitrophenol to burned or abraded skin is dangerous even for nonsensitive persons since many deaths have been reported from its application," no reports of human fatalities resulting from 2,4,6-TNP exposure were found in the literature. Gleason, et al. (1968) reports the lowest recorded lethal dose for 2,4,6-TNP in humans as 5 mg/kg body weight, however, details of the poisoning episode were not provided. It is reasonable to assume, based on the known toxicity of 2, 4,6-TNP in experimental animals, that exposure to sufficient amounts of the compound would result in lethal effects in humans. The limited acute toxicity information for experimental animals has been compiled and presented as Table 9.

Following acutely lethal doses of 2,4,6-TNP, dogs die from respiratory paralysis (Dennie, et al. 1929). Autopsy results demonstrate the presence of yellow staining of the subcutaneous fat, the lungs, the intestines and the blood vessels. Swelling of the liver and glomerulitis of the kidneys were also seen.

The major effect of non-lethal doses of trinitrophenol (TNP) appears to be an allergic or irritative dermatitis (Anon. 1937; Ehrenfried, 1911). According to Dennie, et al. (1929) about four percent of people treated with TNP are sensitive and develop a local dermatitis. Reactions may also appear in unexposed areas. An intense itching and burning, printus, skin eruptions, and irritability are common. Skin eruptions are characterized by irregular-shaped macules, popules, vesicles, blebs, excoriations, and edema, as well as dried yellow crusts which are sources of reabsorption. In the maculopapular stage, a purplish-yellow color is characteristic, even in distant lesions.

More severe reactions can lead to diffuse, often severe erythema and desquamation of affected areas (Sulzburger and Wise, 1933; Am. Conf. Gov. Ind, Hyg., 1971). The reaction may last from several weeks to almost a year (Sulzburger and Wise, 1933).

TABLE 9

Acute Toxicity of Trinitrophenol Isomers[a]

Species	Dose (mg/kg)	Route of Administration	Effects	References
		2,4,6-Trinitrophenols		
Dog	100-125	S.C.	Lethal Dose	Dennie, et al. 1929
Dog	60	S.C.	MLD	Spector, 1956
Dog	60	?	MLD	von Oettingen, 1949
Rabbit	120	Oral	Lethal Dose	von Oettingen, 1949
Frog	200	S.C.	Lethal Dose	Windholz, 1976
Frog	200-300	S.C.	MLD	Spector, 1956
Cat	500[b]	Oral	Lethal Dose	von Oettingen, 1949
Human	5	Oral	Lethal Dose	Gleason, et al. 1968

[a] Acute toxicity data for trinitrophenol isomers other than 2,4,6-TNP were not found.

[b] Total dose in milligrams.

Effects on the skin are apparent at concentrations well below those necessary for oral systemic poisoning. Of 71 individuals exposed at concentrations of 0.0088 to 0.1947 mg/m^3, dermatitis developed only among those exposed to the lower concentrations. Desensitization or adaptation reactions may occur (Am. Conf. Gov. Ind. Hyg., 1971).

Guinea pigs tested for allergic reactions gave similar results (Landsteiner and DiSomma, 1940; Maguire and Chase, 1972; Maguire, 1973; Chase and Maguire, 1972). Using a split-adjuvant method of sensitization, reactions have been noted at concentrations of less than one percent, with weaker solutions often giving stronger reactions. A boosting effect was also noted on subsequent tests with sensitized animals (Maguire and Chase, 1972).

Sub-lethal doses of less than or equal to 50 mg/kg body weight in dogs have resulted in transitory changes in the kidney which include glomerulitis and involvement of TNP ranging from cloudy swelling to gelatinous degeneration. The liver also showed a cloudy swelling with no staining while the lungs were stained brownish-yellow in some animals (Dennie, et al. 1929).

Other reactions in humans include central nervous system effects resulting in temporary impairment of speech, memory, walking, and reflexes (Dennie, et al. 1929) and microscopic hematuria caused by ingestion of TNP (2 to 20 mg/l) in water distilled from sea water (Harris, et al. 1946).

Although it is not possible to estimate precisely either the TNP water levels or duration of exposure necessary to induce hematuria, Harris, et al. (1946) detected levels of 10 µg/l and 20 µg/l in drinking water aboard two ships at the time of the hematuria outbreak.

Crenation of intact erythrocytes by 1 mM 2,4,6-TNP has been noted by Sheetz and Singer (1976) with lysing occurring at higher concentrations. 2,4,6-TNP has also been observed to affect glycolysis in human red blood cells in vitro by affecting ATP production and consumption, although the exact mechanism has not been determined (Vestergaard-Bogind and Lunn, 1977). Pugh and Stone (1968) noted that 6 to 11 mg/kg 2,4,6-TNP administered intravenously to anesthetized dogs results in a moderate increase in bile flow and a rise in body temperature. The clinical significance of these effects is unknown.

Synergism and/or Antagonism

Information on synergistic or antagonistic effects involving 2,4,6-TNP is scant in the literature. An interesting study by Huidobro (1971) demonstrated that administration of 50 mg/kg of 2,4,6-TNP 30 minutes before the administration of a number of analgesic drugs resulted in a significant increase area of analgesia induced by the opioids and the minor analgesics employed. 2,4,6-TNP did not, itself, evoke analgesia. The compounds tested included: morphine, meperidine, methadone, pentazocine, aminopyrine, sodium salicilate, and etonitazene. The investigators suggested that the elimination or

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metabolism of the analgesics may be modified by an effect of 2,4,6-TNP on enzymatic systems. However, further investigation is needed to definitively answer this question.

Teratogenicity

No information on possible teratogenic effects of 2,4,6-TNP was found.

Mutagenicity

Streptomycin-independent mutants were induced in streptomycin-requiring *E. coli* B/Sd-4 after preincubation in the presence of 0.18 percent 2,4,6-TNP for three hours before plating on streptomycin free agar (Demerec, et al. 1951). Yoshikawa, et al. (1976) reported that 2,4,6-TNP was capable of inducing mutations in *Salmonella*, when tested in a system which included microsomal activation. In contrast, Auerbach and Robson (1947) failed to demonstrate sex-linked lethals in *Drosophila* after bathing the eggs in an aqueous solution of 2,4,6-TNP. Other data on possible mutagenic properties of 2,4,6-TNP were not found.

Carcinogenicity

Data addressing possible carcinogenic effect of 2,4,6-TNP were not found.

DINITROCREOSOLS

Mammalian Toxicology and Human Health Effects

EXPOSURE

Introduction

Dinitro-ortho-cresol is a yellow crystalline solid derived from o-cresol. There are six possible isomers but the 4,6-dinitro-o-cresol isomer is the only one of any commercial importance. In fact, a comprehensive search of the literature failed to reveal information on any of the other five dinitro cresol isomers.

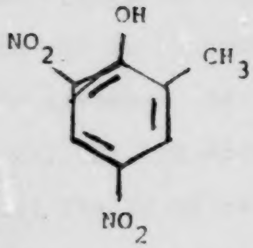
4,6-dinitro-o-cresol (hereafter referred to as DNOC) is produced either by sulfonation of o-cresol followed by treatment with nitric acid or by treatment of o-cresol in glacial acetic acid with nitric acid at low temperature. Some important chemical and physical properties of DNOC are shown in Table 10.

TABLE 10

Properties of 4,6-Dinitro-o-cresol

Molecular Weight	198.13
Appearance	Yellow Solid
Melting Point	85.8°C
Vapor Pressure	0.000052 mm Hg at 20°C
Water Solubility	100 mg/l at 20°C
pKa	4.46

Dinitrocresols



4,6-dinitro-o-cresol

An excellent review of the toxicological effects of DNOC on human and laboratory animals has recently been published by the National Institute for Occupational Safety and Health (1978). In view of the comprehensive coverage of both English and foreign language literature, no attempt will be made to duplicate this impressive effort within this criterion document. Key papers used for criterion formulation will be cited, where appropriate, and frequent reference to the NIOSH review will be used where the available literature does not contain information directly relevant to criteria formulation.

DNOC usage in the U.S. has declined in recent years because the compound is highly toxic to plants in the growing stage and nonselectively kills both desirable and undesirable vegetation. Additionally, the compound is highly toxic to humans and is considered one of the more dangerous agricultural pesticides.

The Environmental Protection Agency has no record of DNOC being currently manufactured in the United States for use as an agricultural chemical. Imports of DNOC have also decreased in recent years; from 217,899 lbs. in 1972 to 146,621 lbs. in 1973 and then to 30,442 lbs. in 1976 (Natl. Inst. Occup. Safety Health, 1978). Since DNOC is not manufactured in the U.S., pesticide formulators and sprayers are the major groups with potential occupational exposure to DNOC.

DNOC is used primarily as a blossom-thinning agent on fruit trees and as a fungicide, insecticide, and miticide on fruit trees during the dormant season. NIOSH (1978) estimates that 3,000 workers in the U.S. are potentially exposed to DNOC. In view of the small amount of DNOC used in the U.S., exposure of the general public is expected to be minimal.

Ingestion from Water

Monitoring data on the presence of DNOC in ambient water are not available. An unspecified amount of DNOC was detected in the wastewaters of Fison's Pest Control Limited in Harston, Cambridge, England (Jenkins and Hawkes, 1961). Webb, et al. (1973) detected 18 mg/l DNOC in the wastewater of a specialty chemical plant. The extent to which human exposure to DNOC results from the ingestion of contaminated water is unknown.

Ingestion from Foods

No data are available on the presence or absence of DNOC residues in food for human consumption. Since the primary usage of the compound involves treatment of fruit trees during the dormant season, it appears unlikely that contamination of human food stuffs would occur to any large extent.

No measured steady-state bioconcentration factor (BCF) is available for any nitrophenol; however, an estimated value can be derived by using a log equation (Veith, et al., Manuscript) based upon the octanol-water partition coefficient. Thus, the weighted average BCF for DNOC and the edible portion of all aquatic organisms consumed by Americans is 7.5 (Table 2A).

Inhalation

An evaluation of the literature (Natl. Inst. Occup. Safety Health, 1978) indicates that occupational injury and disease associated with exposure to DNOC results primarily from inhalation of, and skin contact with, the aerosol form. A large number of human intoxications, including fatalities, have been reported resulting from such DNOC exposure. Persons at risk include those manufacturing, formulating, or applying the compound as an aerosol. Inhalation esposure to the general public is expected to be minimal although data addressing this point are not available

Dermal

As mentioned in the preceeding section, occupational intoxication by exposure to DNOC has occurred as a result of inhalation and dermal exposure where the compound is manufactured, formulated or applied. Dermal exposure of the general public is considered unlikely, however, direct data bearing on this point were not found.

PHARMACOKINETICS

Absorption

DNOC is readily and rapidly absorbed through the skin, the gastrointestinal tract and respiratory tract in humans (Natl. Inst. Occup. Safety Health, 1978). Although most cases of occupational intoxication resulting from DNOC exposure contain both a respiratory and a dermal component, human intoxication has been reported as a result of dermal contact to DNOC alone.

In a report from the Russian literature (Buchinskii, 1974; reviewed by NIOSH, 1978) a four-year-old boy was fatally intoxicated after a rash had been treated with 50 g of an ointment to which 25 percent DNOC was added by mistake. Stott (1956) reported two cases of DNOC poisoning resulting from skin absorption. The two men were involved in the cleaning and maintenance of aircraft booms used to spray solutions of DNOC. Since neither man worked near the actual operation, and both denied blowing into the spray jet to clean them, Stott (1956) concluded that the major route of exposure was skin contact.

Work by Harvey, et al. (1951) indicates that DNOC is rapidly absorbed by the human gastrointestinal tract. These investigators described the effects of DNOC taken orally by five male volunteers. It was noted the DNOC levels in the blood increased steadily after administration and were maximal from two to four hours after ingestion. Van Noort, et al. (1960; reviewed by NIOSH, 1968) investigated the effectiveness of personal protective equipment used by 24 sprayers in Holland. Serum DNOC levels and the quantity of DNOC used were determined in a three-week spraying period. Protective equipment ranged from usage of full body covering and masks to individuals who failed to use any type of protective equipment. Their findings indicated that both inhalation of, and dermal contact with, DNOC can lead to an appreciable absorption into the blood stream.

Experimental animal studies, reviewed by NIOSH (1978), also have confirmed the toxicity of DNOC in humans exposed by the oral, inhalation, and dermal routes.

Distribution

Whether absorption of DNOC occurs through the skin, gastrointestinal tract, or respiratory tract, the compound is transported in and distributed by the blood (Natl. Inst. Occup. Safety Health, 1978). Harvey, et al. (1951) described the effect of DNOC taken orally by five male volunteers. Capsules containing 75 mg of pure DNOC were administered daily for five consecutive days amounting to a dose of 0.95 to 1.27 mg/kg/day. The concentration of DNOC in the blood increased in the first three to four days and reached concentrations of 15 to 20 mg/kg. After concentrations of 15 to 20 mg/kg had been obtained, additional doses appeared to cause temporary high blood concentrations which were associated with toxic symptoms.

Blood analysis of humans displaying symptoms of DNOC toxicity has invariably revealed concentrations exceeding 10 mg/kg (Natl. Inst. Occup. Safety Health, 1978).

In studies conducted to determine the kinetics of absorption and distribution, DNOC has not been shown to accumulate in the blood of various animal species (King and Harvey, 1953a; Parker, et al. 1951). In rats and rabbits that were given two or more daily injections of DNOC subcutaneously, serum levels on succeeding days were no higher than they were 24 hours after the first dose (Parker, et al. 1951). Serum levels in dogs rose for the first three days but then decreased despite the administration of two additional doses.

DNOC is more rapidly eliminated from the blood of animals than from the blood of humans (King and Harvey, 1953b; Parker, et al. 1951; Harvey, et al. 1951). Within a 24-hour period following a single subcutaneous injection of DNOC, elimination from the serum of rabbits was nearly complete. Four days were necessary for serum clearance in rats and cats, while six days were required for elimination from the serum of dogs (Parker, et al. 1951). DNOC accumulated only slightly in the blood when given to rats by stomach tube or i.p. injection and did not accumulate in the blood of rabbits after administration by stomach tube (King and Harvey, 1953a).

The accumulation of DNOC in the blood of humans following DNOC exposure has been well documented (Harvey, et al. 1951; Bidstrup, et al. 1952). The accumulative effect may reflect the binding of DNOC with albumin in the blood and a subsequent slow rate of excretion in humans (Harvey, et al. 1951).

DNOC is slowly eliminated from humans. The investigations by Harvey, et al. (1951) indicated detectable amounts of DNOC in the blood (1 mg/kg) as long as 40 days following the last of five consecutive daily oral doses in human volunteers. Another study (Van Noort, et al. 1960; reviewed by NIOSH, 1978) showed that it took two to eight weeks for DNOC to be cleared from the serum.

Parker, et al. (1951) studied the tissue distribution of DNOC following subcutaneous injection in the rat. They noted that a single dose of 10 mg/kg DNOC produced very high levels in the serum (100 mg/l at 30 min) but no accumulation in other tissues was detected. The lungs and heart contained the high levels of DNOC but the investigators postulated that these levels were the highest due to the high blood content of these organs. The investigators calculated that within 30 minutes of the injection, 83 percent of the DNOC that could be accounted for was present in the blood. Six hours after the injection 0.37 mg of the 1.5 mg dose of DNOC could be accounted for, of which 72 percent was in the blood.

DNOC content of a number of tissues was determined in rats receiving a single subcutaneous injection of the compound (Parker, et al. 1951). The results, presented as Table 11, clearly indicate the DNOC failed to accumulate in the tissues.

TABLE 11

DNOC Content of Blood and Tissues[b] of Rats Killed at Intervals After Subcutaneous Injection of One Dose of 1.5 mg DNOC[a]

Time After Injection	Serum (mg/l)	Brain	Spleen	Kidney	Liver	Muscle	Heart	Lung
30 mins.	100	1.5	4.0	7.5	14.0	0.5	8.0	18.0
1 hr.	89	3.5	4.0	7.5	12.0	2.0	13.5	20.0
2 hrs.	97	2.0	4.5	11.0	10.5	0.0	19.0	20.5
3 hrs.	93	4.0	8.0	11.0	11.5	3.5	14.0	15.5
4 hrs.	79	3.5	3.0	4.5	13.5	0.5	13.0	14.0
5 hrs.	76	2.0	4.0	4.5	8.5	2.0	14.0	14.5
6 hrs.	45	3.0	1.5	7.5	8.5	1.5	10.5	30.0

[a] Source: Parker, et al. 1951.

[b] DNOC content of tissue mg/kg net weight.

In another experiment Parker, et al. (1951) failed to detect significant DNOC accumulation in liver or kidney tissue of rats after 40 successive daily injections of 20 mg/kg DNOC.

In a single study reviewed by NIOSH (1978) Sovljanski, et al. (1971) discussed tissue distribution of DNOC in humans. Autopsy results of two suicide victims, by ingestion of DNOC, yielded detectable DNOC in the stomach, intestines, liver, kidneys, heart, and brain, with the stomach containing the greatest amount. Neither blood DNOC levels nor quantitative data on tissue levels were reported.

Steer (1951), on the other hand, demonstrated that the tissues of a fatal case of DNOC poisoning contained no more than 5 mg/kg of DNOC and many contained 1 mg/kg or less.

According to King and Harvey (1953b) the accumulation of DNOC in man can be explained in two ways; either the detoxification and excretion are very slow or there is some storage of DNOC in body tissues. Based on their calculation of excretion kinetics in man, the investigators suggested that detoxification and excretion of DNOC are inefficient and slow in humans.

None of the available data suggest significant accumulation of DNOC in specific tissues of humans or experimental animals (Natl. Inst. Occup. Safety Health, 1978).

Metabolism

The metabolism of DNOC in humans has not been studied. Several investigators have conducted experiments to determine the fate of DNOC after its administration to animals, however.

Truhaut and De Lavaur (1967; Reviewed by NIOSH, 1978) reported on the metabolism of DNOC in rabbits. Following the administration of DNOC by gastric intubation into rabbits, both DNOC and 6-amino-4-nitro-o-cresol were detected in liver, kidney, brain, and urine of animals. 4-amino-6-nitro-o-cresol was not detected in the animals. It was concluded by the investigators that the ratio of 6-amino-4-nitro-o-cresol to DNOC in the tissue and urine was a function of the dose of DNOC administered to the animal. When a low dose of DNOC was administered, very little 6-amino-4-nitro-o-cresol was detected in either the urine or tissues. The authors considered the metabolism of DNOC to 6-amino-4-nitro-o-cresol a detoxification mechanism that plays an important role only when a toxic dose of DNOC is administered. They further suggested that the ratio of 6-amino-4-nitro-o-cresol to DNOC might be a useful indicator in evaluation of the severity of exposure to DNOC.

The metabolic fate of DNOC in rabbits was also investigated by Smith, et al. (1953). Following administration of 20 to 30 mg/kg DNOC to rabbits by stomach tube, urinary metabolites were identified by paper chromatography and spectrophotometry. Less than 20 percent of the dose was recovered in the urine in two days. Between 5 and 5.5 percent was detected as free DNOC, and 0.7 percent as DNOC conjugates. The conjugates were not characterized by the investigators. Most of the urinary metabolites (about 12 percent of the dose) were derivatives of 6-amino-4-nitro-o-cresol. About 1.5 percent of the dose was excreted as

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6-acetamido-4-nitro-o-cresol, and 9 to 10.5 percent as the hydroxyl group conjugate. Traces of 6-amino-4-nitro-o- cresol, 4-amino-6-nitro-o-cresol, and 3-amino-5- nitro- salicylic acid were also detected.

Since the detoxification and excretion of DNOC in man are very slow compared with rats or rabbits (King and Harvey, 1953b), the applicability of the experimental animal detoxification mechanism to the human situation is questionable. The elucidation of DNOC detoxification mechanism in humans awaits further investigation.

Excretion

Available data indicate that DNOC is rapidly excreted following administration to experimental animals. Parker, et al. (1951) found that DNOC injected subcutaneously disappeared from the blood at various rates in different species. Single 10 mg/kg doses of DNOC were administered subcutaneously to an unspecified number of dogs, cats, rabbits, and rats. DNOC given in one injection was completely eliminated from the serum of rabbits within 24 hours, while blood DNOC levels were between 30 and 40 mg/l in the rats, cats, and dogs at this time. It took four days for DNOC blood levels to fall to zero in rats and cats, and six days in dogs. The half-time for elimination of DNOC from the blood after a single injection of 10 mg/kg DNOC was approximately three hours in the rabbit, 15 hours in the rat, 20 hours in the cat, and 36 hours in the dog.

Lawford, et al. (1954) reported that animals eliminated DNOC from the blood in the following descending order of efficiency: mouse, rabbit, guinea pig, rat, and monkey.

DNOC is eliminated in the blood of animals faster than it is from the blood of humans (King and Harvey, 1953b; Parker, et al. 1951). King and Harvey (1953b) calculated the half-time for elimination of DNOC from the blood of rats, rabbits, and humans. The values were 28.5 hours, 6.6 hours, and 153.6 hours, respectively.

Pollard and Filbee (1951) reported on the urinary excretion of DNOC from a seriously poisoned man in Great Britain. The man was admitted to the hospital and full biochemical investigations were carried out immediately after admission. The man recovered almost totally from the poisoning episode within five days. However, DNOC levels of 4 mg/l were still detected in the blood one month following the exposure. Blood DNOC level was reported to fall in an exponential fashion.

Van Noort, et al. (1960; Reviewed by NIOSH, 1978) measured the serum DNOC levels in ten sprayers on a weekly basis for two months after the spraying period ended. They found the DNOC was eliminated from the serum slowly and that the rate varied from individual to individual. Two to eight weeks elapsed before DNOC was cleared completely from the serum of these workers. The amount of time needed for DNOC to be totally eliminated was directly related to the quantity of DNOC in the serum on the last day of exposure.

In experiments where DNOC was orally administered to five human volunteers, Harvey, et al. (1951) demonstrated that DNOC, absorbed by ingestion at intervals of 24 hours, accumulates in the human body and is excreted slowly. Forty days after the last dose of DNOC was administered by mouth, 1 to 1.5 mg/l DNOC was still present in the blood.

The experimental evidence suggests, therefore, that a substantial difference in the excretion patterns of humans vs. experimental animals exists. Since storage of DNOC in the tissues of humans has not been reported, it is concluded that slow and inefficient detoxification or excretion probably occurs in humans.

Occupational studies (Natl. Inst. Occup. Safety Health, 1978) have long utilized serum levels of DNOC in order to assess when humans are exposed to dangerous amounts of the compound. A review of the literature (Natl. Inst. Occup. Safety Health, 1978) indicates that workers with DNOC concentrations of 40 mg/kg of whole blood (approximatley 80 mg/l of serum) or greater will most likely develop toxic effects. In addition, in the concentration range between 20 and 40 mg/kg of whole blood (probably because of variation in individual susceptibility) some workers are affected and others show no adverse effects. Most workers with blood DNOC levels below 20 mg/kg are not affected, although, again because of individual susceptibility, some exhibited mild effects. The blood level of 20 mg/kg has been used as a maximum permitted level for industrial or agricultural workers utilizing the compound during their employment.

Bidstrup, et al. (1952) recommended that a person should be removed from further contact with DNOC for at least six weeks if the blood level eight hours after the last exposure was 20 mg/kg or higher.

Other data on the elimination of DNOC from humans were not found.

EFFECTS

Acute, Sub-acute, and Chronic Toxicity

The acute toxic dose of DNOC with different routes of administration, has been determined for a number of different experimental animal species. These data have been compiled and presented in Table 12.

Although the available human toxicity data do not permit the calculation of the acute lethal dose for DNOC in humans, it has been estimated (Fairchild, 1977) that 5 mg/kg may prove lethal to humans.

A large number of occupational and nonoccupational poisonings of humans by DNOC have been reviewed by NIOSH (1978). The available literature concerning humans indicates that DNOC may be absorbed in acutely toxic amounts through the respiratory and gastrointestinal tracts and through the skin, and that it accumulates in the blood. Individuals exposed to DNOC by these routes usually demonstrate signs of increased metabolism. Symptoms of poisoning include profuse sweating, malaise, thirst, lassitude, loss of weight, headache, a sensation of heat, and yellow staining to the skin, hair, sclera, and conjunctiva.

In addition to the effects associated with increased metabolism, other effects occasionally reported in humans poisoned by DNOC included kidney damage, diarrhea, unspecified

TABLE 12

Acute Toxicity of 4,6-Dinitro-o-creosol

Species	Dose (mg/kg)	Route of Administration	Effects	References
Mouse	187	Dermal	LD 50	Arustamyan, 1972; Reviewed by NIOSH, 1978
Rabbit	1000	Dermal	LD 50	Burkatskaya, 1965; Reviewed by NIOSH, 1978
Guinea Pig	500	Dermal	100% Lethal	Spencer, et al. 1948
Rat	85	Oral	LD 50	Burkatskaya, 1965; Reviewed by NIOSH, 1978
Rat	30	Oral	MLD	Ambrose, 1942
Rat	40	Oral	100% Lethal	Ambrose, 1942
Rat	30	Oral	LD 50	Spencer, et al. 1948
Mouse	47	Oral	LD 50	Burkatskaya, 1965; Reviewed by NIOSH, 1978
Mouse	16.4	Oral	LD 50	Arustamyan, 1972; Reviewed by NIOSH, 1978
Hare	24.8	Oral	LD 50	Janda, 1970; Reviewed by NIOSH, 1978
Cat	50	Oral	LD 50	Burkatskaya, 1965; Reviewed by NIOSH
Pheasant	8.4	Oral	LD 50	Janda, 1970; Reviewed by NIOSH, 1978
Partridge	8.3	Oral	LD 50	Janda, 1970; Reviewed by NIOSH, 1978
Rat	26-39	S.C.	LD 50	Harvey, 1952
Rat	20	S.C.	MLD	Ambrose, 1942
Mouse	24.2	S.C.	LD 50	Parker, et al. 1951
Rat	24.6	S.C.	LD 50	Spector, 1956
Goat	50	S.C.	LD 50	Ambrose, 1942
Dog	15	I.V.	LD	Spector, 1956
Dog	5	I.M.	LD	Spector, 1956
Dog	10	I.P.	LD	Spector, 1956
Pigeon	5	I.M.	LD	Spector, 1956

changes in the gastrointestinal tract, in the cardiovascular system, and in the peripheral blood and central nervous systems.

It is generally believed that the toxic effects of DNOC result from its ability to uncouple the oxidative phosphorylation process. DNOC is an extremely potent uncoupler of oxidative phosphorylation. At a biochemical level, this effect results in the decreased formation of adenosine triphosphate (ATP) and a resulting inhibitory effect of enzyme reactions requiring ATP. Such a toxicant is expected to have extreme and profound effects on all tissues where the concentration of the chemical is high enough to severely affect oxidative phosphorylation. Since energy generated in the body cannot be converted to its usual form (ATP) in the presence of DNOC, it is released as heat instead, causing many of the commonly observed signs and symptoms of DNOC toxicity.

Several investigations have correlated blood DNOC levels with the severity of toxic effects in humans (Harvey, et al. 1951; Bidstrup, et al. 1952; Pollard and Filbee, 1951) and have shown that, unlike the situation in animals, DNOC accumulates in the blood of humans. Accumulation is believed to occur as a result of DNOC binding to albumin in the blood (Harvey, et al. 1951). In one of the few cases where DNOC in the blood of a poisoned human was monitored throughout his recovery period, (Pollard and Filbee, 1951) the severity of the symptoms decreased as blood levels of DNOC decreased. Data on blood DNOC levels in humans and the accompanying effects are compiled and presented as Table 13. The data show that workers with DNOC concentrations of 40 mg/kg of whole blood (approximately 80 mg/l of serum) or greater will most likely develop toxic effects. In the concentration range between 20 and 40 mg/kg of whole blood, some workers are affected and others show no adverse effect (probably because of differences in individual susceptibility). Most individuals with blood levels of DNOC below 20 mg/kg were not affected, although some exhibited mild effects. As the data in Table 4 suggest, most investigators have concluded that blood DNOC levels are associated with the severity of intoxication in humans (Natl. Inst. Occup. Safety Health, 1978).

In comparing studies on blood DNOC levels, certain precautions must be taken when correlating the results. It has been reported that over 90 percent of the DNOC detected in the blood is found in serum (Parker, et al. 1951) and that most of this amount is bound to albumin in humans (Harvey, et al. 1951). A comparison of numerically similar blood DNOC levels expressed as weight/volume of serum with those expressed as weight/weight of whole blood can therefore only be done by approximate conversions. Any given DNOC serum level will have a lower value when expressed as the amount of whole blood.

It is impossible to develop a dose response relationship for occupational DNOC poisoning in humans since air concentrations of DNOC are rarely reported and the exposure time of poisoned individuals is highly variable. In most cases of human poisoning total exposure amounts can only be estimated.

TABLE 13

Relationship of Blood DNOC Levels and Effects in Humans[a]

Route of Exposure	No. of Individuals and Occupation	Blood DNOC Level (mg/kg)	Effects
Inhalation, Dermal	1 Agricultrual Worker	1000[b,c]	Death
Inhalation, Dermal	1 Agricultural Worker	200[b,c]	Sweating, labored breathing, vomiting
Inhalation, Dermal	1 Agricultural Worker	75	Death
Inhalation, Dermal	1 Agricultural Worker	60	Headache, lassitude, BMR 275%
Inhalation, Dermal	1 Agricultural Worker	60[b,c]	Sweating, headache, labored breathing, fatigue
Inhalation, Dermal	1 Agricultural Worker	55	Unconsciousness
Inhalation, Dermal	2 Agricultural Workers	44-55	Acute Poisoning
Oral	5 Experimental Subjects	40-48	Headache, lassitude, malaise
Inhalation, Dermal	4 Agricultural Workers	20-40[b]	Liver damage
Inhalation, Dermal	5 Agricultural Workers	30-40	No effects
Inhalation, Dermal	6 Agricultural Workers	21-40[b]	Moderate poisoning; recovery period longer than 8 days
Inhalation, Dermal	32 Agricultural Workers	7-37[b]	Mild poisoning; recovery within 8 days
Inhalation, Dermal	1 Agricultural Worker	30[b]	Fever
Inhalation, Dermal	16 Agricultural Workers	20-30	No effects
Inhalation, Dermal	1 Agricultural Worker	25[b]	Kidney damage
Inhalation, Dermal	21 Agricultural Workers	10-20	No effects
Inhalation, Dermal	149 Agricultural Workers	<10	No effects
Inhalation, Dermal	4 Agricultural Workers	4-9[b,c]	Sweating, thirst
Inhalation, Dermal	23 Agricultural Workers	1-8[b,c]	No effects
Inhalation, Dermal	1 Agricultural Worker	<5[b,c]	Fatigue
Inhalation, Dermal	2 Manufacturing Workers	10-20	No effects
Oral	5 Experimental Subjects	20	Exaggerated feeling of well-being

[a] Source: Modified from NIOSH, 1978

[b] Reported as mg/l

[c] Serum or Plasma DNOC level

This lack of data makes assessment of a minimum toxic dose for humans extremely difficult. Several studies however, where the oral toxicity of DNOC has been assessed in humans, shed some light on this question.

Harvey, et al. (1951) orally administered DNOC to five male volunteers and discussed both the resulting blood levels and toxic effects seen in the volunteers. Each man was given capsules containing 75 mg of pure DNOC daily for five consecutive days, amounting to a dose of 0.92 to 1.27 mg/kg/day. The men experienced an exaggerated sense of well-being when blood levels were about 20 mg/kg. Headache, lassitude, and malaise were associated with DNOC blood levels of 40 to 48 mg/kg. Although individual variation was evident in these experiments, it is obvious that chronic administration of 1 mg/kg/day DNOC to healthy humans may result in signs of toxicity. The exaggerated sense of well-being described by Harvey, et al. (1951) is a typical sign of impending toxic effects among agricultural workers exposed to DNOC.

DNOC was introduced in 1933 as an alternative to dinitrophenol for the treatment of obesity (Natl. Inst. Occup. Safety Health, 1978). Many poisonings, and some deaths, resulting from overdoses were reported, as well as the development of cataracts in some patients, months after they had stopped taking DNOC. Some patients developed symptoms of DNOC poisoning at the accepted theraputic dose level. Signs and symptoms of DNOC intoxication including thirst, fatigue, excessive sweating, decreased appetite, and elevated basal metabolic rates, appeared in three persons who had taken as little as 0.35 to 1.5 mg/kg/ day of DNOC for up to nine weeks (Plotz, 1936). Hunter (1950; reviewed by NIOSH, 1978) noted that, although, less than one percent of those individuals treated with DNOC developed complications, he considered the difficulty of setting a safe dose for each individual to be the reason that its use as an aid to weight loss was discontinued.

Although DNOC is considered a cumulative poison in humans, probably as a result of slow metabolism and inefficient excretion, true chronic or subacute effects (with the possible exception of cataract formation) have never been reported in either human or experimental animals. Signs and symptoms of toxicity occur when the total body burden exceeds a threshold level. The toxic effects noted after either acute or chronic administration are similar in quality and their severity appears to be correlated with DNOC blood levels (and by inference, total body burden). It is generally agreed that the toxic manifestations of DNOC result from its potent effects on metabolism (Natl. Inst. Occup. Safety Health, 1978).

Several long-term studies designed to determine dietary levels of DNOC needed to cause toxic symptoms in experimental animals have been conducted. Spencer, et al. (1948) maintained rats on a diet containing DNOC for six months. Growth curves, periodic blood counts, analyses of urea-N, organ weights, and histopathological examinations were carried out on all animals. No adverse effects on these parameters were detected among rats fed on diets containing 100 mg DNOC/kg food. Higher concentrations in the diet produced effects that may be attributed to the action of DNOC as a metabolic stimulant. Such effects included: weight loss or poor weight gain, marked emaciation, a hungry, thin, and unkempt appearance, and minor histopathological effects on the liver, kidneys, and spleen at the highest dose level (1000 mg DNOC/kg food). For water, a concentration of approximately one-half the dietary intake will result in the equivalent dosage on a body weight basis (assuming a fluid intake two times the dry matter intake). Thus, the no observable effect level for DNOC in rats if all DNOC were derived from drinking water would be 200 mg/l.

In a similar study Ambrose (1942) reported no observable effect on rats fed diets containing 63 mg DNOC/kg food for 105 days. At DNOC levels of 125 mg/kg food, 60 percent of the animals died. At necropsy and histopathological examination, the tissues of all rats receiving the drug for 30 days or more failed to show any characteristic lesions that could be ascribed to the drug. The calculated no effect level for DNOC in drinking water is 126 mg/l.

When DNOC was administered in the diet of rats by Parker, et al. (1951) poisoning was only observed when the calculated daily intake of the drug greatly exceeded the single lethal dose. At a level of 200 mg DNOC/kg food, rats grew normally over an observed period of 18 weeks.

A Federal workplace environmental limit of 0.2 mg/m^3 for DNOC has been recommended by NIOSH (1978). The limit was based on the following considerations: A study from the Russian literature (Burkatskaya, 1965; reviewed by NIOSH, 1978)

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documented the lowest airborne DNOC levels found in the literature associated with health effects in humans. Exposure to airborne DNOC at concentrations that averaged 0.9 mg/m^3 produced unspecified changes in the cardiovascular system, the central and autonomic nervous systems, the gastrointestinal tract, and the cell pattern of the peripheral blood of workers involved in manufacturing and applying DNOC. In agricultural workers exposed to DNOC at an average concentration of 0.7 mg/m^3, slight unspecified changes in the blood and autonomic nervous system were observed.

Another study (Batchelor, et al. 1956) revealed that agricultural sprayers exposed to an airborne DNOC concentration of about 0.23 mg/m^3 failed to demonstrate adverse effects of the compound. No symptoms of poisoning were observed and blood DNOC levels were well below those associated with toxic effects.

In the study by Burkatskaya (1965; reviewed by NIOSH, 1978) the effect of airborne DNOC on cats was examined. Cats exposed at 0.2 mg/m^3 for two or three months had slightly increased body temperatures and leucocyte counts and decreased hemoglobin concentrations, erythrocyte counts, and catalase and peroxidase activity. The changes, which were characterized as slight and transient, occurred after one to two weeks but further exposure produced no additional effects.

The report by NIOSH (1978) concluded "since only slight effects were seen in workers exposed to DNOC at an average concentration as low as 0.7 mg/m^3 for an unspecified duration, and since short-term exposure at 0.2 mg/m^3 had no lasting effect on cats," NIOSH recommends that the current Federal workplace environmental limit of 0.2 mg/m^3 be retained.

It is possible to calculate the anticipated daily exposure of a 70 kg human male exposed to 0.2 mg/m^3 DNOC for an eight-hour period. If one assumes the average minute volume was 28.6 liters of air/minute (average minute volume for a man doing light work--NIOSH, 1978) the anticipated daily exposure is 39 µg/kg/day.

If one assumes that absorption of DNOC across the respiratory tract is identical to gastrointestinal absorption, and that a 70 kg human male consumes 2.0 liters of water daily, the following calculation indicates the maximum allowable levels of DNOC in drinking water based on the NIOSH air standard values.

$$39 \text{ µg/kg/day} \times 70 \text{ kg} = 2.73 \text{ mg/day}$$

$$\frac{2.75 \text{ mg/day}}{2 \text{ l/day}} = 1.38 \text{ mg/l}$$

Although NIOSH (1978) states "the standard was not designed for the population-at-large, and any extrapolation beyond the occupational environment is not warranted," development of a base-line level for chronic human effects using the same data used by NIOSH appears to be a reasonable way in which to approach the development of a water criteria.

In summary, daily human exposure to 0.35 mg/kg DNOC may result in signs of intoxication in humans. Some persons develop cataracts as a result of chronic exposure to DNOC, but the no effect level for this effect cannot be calculated. Although true "chronic" effects of DNOC have never been documented, the compound accumulates in the human body and toxic symptoms may develop when blood levels exceed 20 mg/kg. Such symptoms have been observed in humans receiving as little as 0.35 mg/kg/day over a period of several weeks. The no observable effect level for rats in long term feeding studies has been variously reported as 63 mg/kg food, 100 mg/kg food, and 200 mg/kg food. Based on the available human and experimental animal data, NIOSH (1978) has recommended a Federal workplace limit of 0.2 DNOC/m^3 air. Based on an estimate of human exposure for an eight-hour work shift, it was calculated that a drinking water level of 1.4 mg/l would result in a similar exposure to the general population.

Synergism and/or Antagonism

No information was found describing synergistic or antagonistic effects associated with DNOC.

Teratogenicity

No information was found regarding the presence or absence of teratogenic properties of DNOC.

Mutagenicity

Andersen, et al. (1972) reported an evaluation of the ability of 110 herbicides, including DNOC, to produce point mutations in histidine-dependent mutants of Salmonella typhimurium, bacteriophage T4, and in two RII mutants of bacteriophage T4. The culture media were prepared by mixing freshly grown cultures of the mutants with soft agar and pouring into petri dishes. After the agar solidified, DNOC was applied to the surface of each plate. They found that the mutation frequency rates produced by DNOC were no greater than the spontaneous rates.

Nagy, et al. (1975) tested DNOC for its ability to induce back-mutations of her^+ and her^- derivatives of E. coli WP2 try-bacteria. DNOC failed to induce reverse mutations in this system.

The difference in growth inhibitions of wild type Proteus mirabilis and the corresponding repair-deficient strain has been used by Adler, et al. (1976) as an indication of DNA damage. Evidence of DNA damage in the presence of DNOC was reported.

Information on the potential mutagenicity of DNOC for mammals is not available.

Carcinogenicity

DNOC has not been tested for carcinogenicity, although Spencer, et al. (1948) failed to report tumor formation in rats maintained on diets containing DNOC for six months. Similarly, no tumors were reported in rats maintained on diets containing DNOC for 105 days (Ambrose, 1942) or 126 days (Parker, et al. 1951).

No further information was found regarding the presence or absence of carcinogenic properties of DNOC.

CRITERION FORMULATION

Existing Guidelines and Standards

No U.S. standards for exposure to the nitrophenols or dinitrocresols in drinking or ambient water have been set.

The following limits for toxic substances in drinking water have been set in the U.S.S.R. (Stofen, 1973):

2-nitrophenol	.06 mg/l
3-nitrophenol	.06 mg/l
4-nitrophenol	.02 mg/l
2, 4-dinitrophenol	.03 mg/l

Based on organoleptic considerations a limit of 0.5 mg/l for 2,4,6-trinitrophenol has been set by the U.S.S.R. (Stofen, 1973).

The maximum air concentration established by the American Conference of Governmental Industrial Hygienists (1971) is 0.1 mg/m^3 for 2,4,6-trinitrophenol and 0.2 mg/m^3 for 4,6-dinitro-o-cresol for an eight-hour exposure (TLV).

The Code of Federal Regulations (40 CFR Part 180) establishes a tolerance of 0.02 mg/kg for residues of 4, 6-dinitro-o-cresol and its sodium salt in or on apples resulting from applications to apple trees at the blossom stage as a fruit-thinning agent.

Current Levels of Exposure

Human exposure to the nitrophenols or dinitro-o-cresols has not been monitored. Unspecified amounts of 4-nitrophenol have been detected in samples of urban ambient particulate matter.

The photochemical reaction between benzene vapor and nitrogen monoxide results in the production of 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, and 2,6-dinitrophenol under laboratory conditions and 4-nitrophenol has been detected in rainwater in Japan. Available data indicate that the general public may be exposed to nitrophenols in the atmosphere when severe photochemical fog conditions develop. Quantitative estimates of such exposures are not possible at the present time.

4-nitrophenol has been detected in the urine of 1.7 percent of the general population at levels as high as .1 mg/l (with a mean urinary level of 10 μg/l).

If it is assumed that urinary residues of 4-nitrophenol reflect direct exposure to the compound, a pharmacokinetic estimate of exposure based on steady-state conditions can be made. The exposure level leading to the 1.7 μg/l residue can be calculated as follows.

$$\text{Exposure} = \frac{(10\ \mu\text{g/l})\ (1.4\ \text{l of urine/day})}{(70\ \text{kg/man}} = 0.20\ \mu\text{g/kg/day}$$

A similar calculation using the maximum urine residue level observed (113 μg/l) gives an exposure of 2.26 μg/kg/day.

These urine levels are not believed to result from direct exposure to 4-nitrophenol, however. A number of widely used pesticides, including parathion, are readily metabolized to 4-nitrophenol in the human body and are believed to be the source of 4-nitrophenol residues in human urine.

Current levels of human exposure to the nitrophenols or dinitrocresols (with the possible exception of 4-nitrophenol) are either very low, non-existent, or have gone undetected. In the absence of data any of the above could be operative.

Special Groups at Risk

The only individuals expected to be at risk for high exposure to the nitrophenols are industrial workers involved in the manufacture of compounds for which the nitrophenols are intermediates. Since picric acid (2,4,6-trichlorophenol) may find some usage as an explosive, germicide, tanning agent, fungicide, tissue fixative, and industrial process material, a higher risk of exposure exists among personnel engaged in such operations.

Although 4,6-dinitro-o-cresol (DNOC) is no longer manufactured in the U.S., a limited quantity is imported and used as a blossom-thinning agent on fruit trees and as a fungicide, insecticide, and miticide on fruit trees during the dormant season. Hence, individuals formulating or spraying the compound incur the highest risk of exposure to the compound.

Basis and Derivation of Criterion

Mononitrophenols	no criterion
Dinitrophenols	68.6 μg/l
Trinitrophenols	10 μg/l
Dinitrocresols	12.8 μg/l

Uncertainty factors used for criteria formulation have been loosely adapted from Drinking Water and Health (Natl. Acad. Sci., 1977).

The organoleptic thresholds for mononitrophenols in water range from 0.24 to 389 mg/l. These levels extracted from the Russian literature are detection thresholds. Acceptability thresholds from the standpoint of human comsumption are not available.

With the exception of a single study abstracted from the Russian literature, data on chronic mammalian effects of the mononitrophenols are absent from the literature.

The Russian investigation (Makhinya, 1969) was reported in abstract form only. Attempts to obtain the full report proved fruitless. The investigators reported distinct cumulative properties of the mononitrophenol isomers in mammals. Threshold levels for effects of mononitrophenols on conditioned reflex activity were reported but details of the experiment including animal species, mode of administration, duration of the experiment, and the exact parameters measured are not available. Hence, it does not seem prudent to develop a criteria based on these results.

In the absence of data on chronic mammalian effects no water criteria for human health can be established for the mononitrophenol isomers at this time.

Information on the dinitrophenol isomers is limited to 2,4-dinitrophenol. Spencer, et al. (1948), in a six-month feeding study with rats demonstrated the no-observable-effect level (NOEL) for 2,4-dinitrophenol to be between 5.4 mg/kg and 20 mg/kg. Taking the lower of the two figures and assuming a 70 kg man consumes 2 liters of water daily and 18.7 grams of contaminated fish having a BCF of 2.4, the NOEL for humans based on the results obtained in rats may be calculated as follows:

$$5.4 \text{ mg/kg} \times 70 \text{ kg} = 378 \text{ mg}$$

$$\frac{378 \text{ mg}}{2 \text{ liters} + (2.4 \times 0.0187) \times 1.0} = 185.3 \text{ mg/l}$$

Based on these calculations no biological effect would be predicted in a man directly ~~or~~ indirectly exposed to ambient water containing 185.3 mg/l 2, 4-DNP.

Experience with the use of 2,4-DNP as an anti-obesity drug in the 1930's indicates that adverse effects, including cataract formation, may occur in humans exposed to as little as 2 mg/kg/day. The drug was frequently used in an uncontrolled manner and the available data do not allow the calculation of a no-adverse-effect level in humans. It is clear, however, that ingestion of 2 mg/kg/day 2,4-DNP for a protracted period may result in adverse effects, including cataracts, in a small proportion of the population. Assuming a 70 kg man consumes 2 liters of water daily and 18.7 grams of contaminated fish having a BCF of 2.4 and assuming 100 percent gastrointestinal absorption of 2,4-DNP, a 2 mg/kg dose of 2,4-DNP would result if ambient water contained 68.6 mg/l of 2,4-DNP.

$$\frac{140 \text{ mg/day}}{2 \text{ liters} + (2.4 \times 0.0187) \times 1.0} = 68.6 \text{ mg/l}$$

These data taken together with the demonstrated bacterial mutagenicity of 2,4-DNP (Demerec, et al. 1951) and the suspected ability of the compound to induce chromosomal breaks in mammals (Mitra and Manna, 1971) suggest that an uncertainty factor of 1,000 should be used in criteria formulation.

The suggested water criterion for 2,4-DNP is, therefore:

$$\frac{68.6\ \text{mg/l}}{1{,}000} = 68.6\ \mu\text{g/l}$$

The available data are insufficient to enable calculation of water criterion levels for the remaining dinitrophenol isomers. For the present, it seems reasonable to assume that the 2,4-dinitrophenol criterion would be appropriate for the other isomers.

Chronic mammalian toxicology data for the trinitrophenols are absent from the literature. An outbreak of microscopic hematuria among shipboard U.S. Navy personnel exposed to 2,4,6-trinitrophenol in drinking water has been reported, however. Although it is not possible to precisely estimate either the 2,4,6-trinitrophenol water level or duration of exposure required for the development of hematuria 2,4, 6-trinitrophenol levels of 10 mg/l and 20 mg/l were detected in drinking water aboard two ships at the time of the outbreak.

Two studies (Demerec, et al. 1951; Yoshikawa, et al. 1976) have demonstrated mutagenic activity of 2,4,6-trinitrophenol in bacterial systems. Auerbach and Robson (1947) failed to detect mutagenic activity in *Drosophila*, however.

Based on the presumed development of hematuria in humans at drinking water levels of 10 mg/l and the evidence indicating mutagenic activity in bacteria, an uncertainty factor of 1,000 is suggested for formulation of the 2,4,6-trinitrophenol water criteria:

$$\frac{10\ \text{mg/l}}{1{,}000} = 10\ \mu\text{g/l}$$

Since available data are insufficient to enable calculation of water criterion levels for the remaining trinitrophenol isomers, it seems reasonable to assume, for the present, that the 2,4,6-trinitrophenol criterion is appropriate for the other isomers.

Although 4,6-dinitro-o-cresol (DNOC) is considered a cumulative poison in humans, probably as a result of slow metabolism and inefficient excretion, true chronic or subacute effects have never been reported in either humans or experimental animals. Since DNOC is not a cumulative poison in experimental animals, extrapolation to humans from long-term animal studies is of questionable value.

The no-observable-effect level (NOEL) for DNOC respiratory exposure in humans has been reported as 0.2 mg/m^3 air (Natl. Inst. Occup. Safety Health, 1978). NIOSH (1978) has, in fact, recommended that the current Federal workplace environmental limit of 0.2 mg/m^3 be retained, based on the available data.

It is possible to calculate the anticipated daily exposure of a 70 kg human male exposed to 0.2 mg/m^3 for an eight-hour period. If one assumes the average minute volume is 28.6 liters of air/minute (average minute volume for a man doing light work--NIOSH, 1978) the anticipated daily exposure is 39 µg/kg/day. Since the NOEL's calculated from long-term experimental animal studies are considerably higher than this value, it will be used as a basis for the suggested water criterion.

If one assumes that absorption of DNOC across the respiratory tract is identical to gastrointestinal absorption, and that a 70 kg human male consumes 2 liters of water daily and 18.7 g of contaminated fish having a BCF of 7.5, the following calculations indicates the maximum allowable levels of DNOC in drinking water based on the NIOSH air standard values:

$$39\ \mu g/kg/day \times 70\ kg = 2.73\ mg/day$$

$$\frac{2.73\ mg/day}{(2/l + (7.5 \times 0.0187) \times 1.0} = 1.28\ mg/l$$

In view of the lack of data indicating chronic effects and the existence of a very recent Federal guideline for human exposure, an uncertainty factor of 100 is chosen for the protection of the general public. The suggested criterion for 4,6-dinitro-o-cresol (and in the absence of adequate data, the other dinitrocresol isomers) is

$$\frac{1.28\ mg/l}{100} = 12.8\ \mu g/l$$

REFERENCES

Adler, B., et al. 1976. Repair-defective mutants of *Proteus mirabilis* as a prescreening system for the detection of potential carcinogens. Biol. Zbl. 95: 463.

Aitio, A. 1973. Glucuronide synthesis in the rat and guinea pig lung. Xenobiotica 3: 13.

Alexander, M., and B.K. Lustigman. 1966. Effect of chemical structure on microbial degradation of substituted benzenes. Jour. Agric. Food Sci. 14: 410.

Ambrose, A.M. 1942. Some toxological and pharmaceutical studies on 4,6-dinitro-o-creosol. Jour. Pharmacol. Exp. Ther. 76: 245.

Amer, S.M., and E.M. Ali. 1969. Cytological effects of pesticides. IV: mitotic effects of some phenols. Cytologia 34: 533.

American Conference of Governmental Industial Hygienists. 1971. Documentation of the threshold limit values for substances in workroon air. Vol. 1. 3rd. ed. Cincinnati, Ohio.

Andersen, K.J., et al. 1972 Evaluation of herbicides for possible mutagenic properties. Jour. Agric. Food Chem. 20: 649.

Anonymous. 1937. Editorial. Jour. AMA 108: 992.

Archer, T.E. 1974. Dissipation of parathion and related compounds from field-sprayed spinach. Jour. Agric. Food Chem. 22: 974.

Arnold, L., et al. 1976. Studies on the modification of renal lesions due to aspirin and oxyphenbutazone in the rat and the effects on the kidney of 2,4-dinitrophenol. Pathology 8: 179.

Arterberry, J.D., et al. 1961. Exposure to parathion: Measurement by blood cholinesterase level and urinary p-nitrophenol excretion. Arch. Environ. Health 3: 476.

Auerbach, C., and J.M. Robson. 1947. XXXIII. - Tests of chemical substances for mutagenic action. Proc. R. Soc. Edinburgh, Sect. B 623: 284.

Batchelor, M.S., et al. 1956. Dinitro-ortho-cresol exposure from apple-thinning sprays. AMA Arch. Ind. Health 13: 593.

Bettman, J.W. 1946. Experimental dinitrophenol cataract. Am. Jour. Ophthal. 29: 1388.

Bidstrup, P.L., et al. 1952. Prevention of acute dinitro-ortho-creosol (DNOC) poisoning. Lancet 1: 794.

Boutwell, R.K., and D.K. Bosch. 1959. The tumor-promoting action of phenol and related compounds for mouse skin. Cancer Res. 19: 413.

Bowman, P. 1967. The effect of 2,4-dinitrophenol on the development of early chick embryos. Jour. Embryol. Exp. Morphol. 17: 425.

Brebion, G., et al. 1967. Studying the biodegradation possibilities of industrial effluents. Application to the biodegradation of phenols. Rev. Inst. Fr. Petrole Ann. Combust. Liquides 22: 1029.

Broadway, D.E., and T.M. Shafik. 1973. Parathion exposure studies. A gas chromatographic method for the determination of low levels of p-nitrophenol in human and animal urine. Bull. Environ. Contam. Toxicol. 9: 134.

Buchinskii, V.I. 1974. Fatal poisoning with dinoc (dinitro-o-creosol). Sub.-Med. Ekspert. 17: 52.

Burkatskaya, E.N. 1965. Maximum permissible concentration of dinitro-o-creosol in air. Gig. Sanit. 30: 30.

Burke, J.F., and M.W. Whitehouse. 1967. Concerning the differences in uncoupling activity of isomeric dinitrophenols. Biochem. Pharmacol. 16: 209.

Burnham, A.K., et al. 1972. Identification and estimation of neutral organic contaminants in potable water. Analyt. Chem. 44: 139.

Burrows, D., and J.C. Dacre. 1975. Toxicity to aquatic organisms and chemistry of nine selected waterborne pollutants from munitions manufacture - a literature review. U.S. Army Med. Bioeng. Res. Div. Lab., Ft. Detrick, Md.

Buselmaier, W., et al. 1976. Comparative investigations on the mutagenicity of pesticides in mammalian test systems. Mutat. Res. 21: 25.

Butler, G.J., and A.H. Bodner. 1973. Health hazard evaluation/toxicity determination. The Boeing Co., Hill Air Force Base, Utah. NIOSH TR-082-74. Natl. Inst. Occup. Safety Health, Cincinnati, Ohio.

Cairns, J., et al. 1976. Aquatic organisms response to severe stress following acutely sublethal toxicant exposure. Water Res. Bull. 12: 1233.

Cameron, M.A.M. 1958. The action of nitrophenols on the metabolic rate of rats. Br. Jour. Pharmacol. 13: 25.

Chase, M.W., and H.C. Maguire. 1972. Picric acid hypersensitivity: Crossreactivity and cellular transfer. Clin. Res. 20: 638.

Chemical Marketing Reporter. 1976. Chemical profile: p-nitrophenol. Chem. Market. Reporter. p. 9.

Cole, J.A. ed. 1974. Groundwater pollution in Europe. Proc. Conf.

Cordle, F., et al. 1978. Human exposure to polychlorinated biphenyls and polybrominated biphenyls. Environ. Health Perspect. 24: 157.

Demerec, M., et al. 1951. A survey of chemicals for mutagenic action on E. coli. Am. Natur. 85: 119.

Dennie, C.C., et al. 1929. Toxic reactions produced by the application of trinitrophenol (picric acid). Arch. Dermatol. Syphilol. 20: 698.

Dietrich, W.C., and R. Beutner. 1946. Failure of o- or p-mononitrophenol to produce cataracts. Fed. Proc. 5: 174.

Dunlop, D.M. 1934. The use of 2,4-dinitrophenol as a metabolic stimulant. Br. Med. Jour. 1: 524.

Eastin, E.F. 1971. Degradation of fluorodifen-1'-^{14}C by peanut seedling roots. Weed Res. 11: 120.

Edsall, G. 1934. Biological actions of dinitrophenol and related compounds: a review. New England Jour. Med. 211: 385.

Ehrenfried, A. 1911. Picric acid and its surgical applications. Jour. AMA 56: 412.

Eiseman, J.L., et al. 1974. Kinetics of in vitro nitro reduction of 2,4-dinitrophenol by rat liver homogenates. Toxicol. Appl. Pharmacol. 27: 140.

Elliott, J.W., et al. 1960. A sensitive procedure for urinary p-nitrophenol determination as a measure of exposure to parathion. Jour. Agric. Food Chem. 8: 111.

El-Refai, A., and T.L. Hopkins. 1966. Parathion absorption, translocation, and conversion to paraxon in bean plants. Jour. Agric. Food Chem. 14: 588.

Fahrig, R. 1974. Comparative mutagenicity studies with Pesticides. Pages 161-181 in R. Montesano and L. Tomatis. eds. Chemical carcinogenesis essays. Proc. workshop on approaches to assess the significance of experimental chemical carcinogensis data for man organized by IARC and the Catholic University of Louvain, Brussels, Belgium. IARC Sci. Publ. No. 10. Int. Agency Res. Cancer, World Health Organization.

Fairchild, E.J., ed. 1977. Agricultural chemicals and pesticides: A subfile of the NIOSH registry of toxic effects of chemical substances. Natl. Inst. Occup. Safety Health, Cincinnati, Ohio.

Feldman, G.L., et al. 1959. Dinitrophenol-induced cataracts in the chick embryo. Jour. Exp. Zool. 140: 191.

Feldman, G.L., et al. 1960. Dinitrophenol-induced cataracts in the avian embryo. Am. Jour. Ophthal. 49: 1168.

Friedman, M.A., and J. Staub. 1976. Inhibition of mouse testicular DNA synthesis by mutagens and carcinogens as a potential simple mammalian assay for mutagenesis. Mutat. Res. 37: 67.

Gabor, M., et al. 1960. Experimental thrombocytosis with o-nitrophenol. Naturwissen-Schaften 49: 470.

Games, H.M., and R.A. Hites. 1977. Composition, treatment efficiency and environmental significance of dye manufacturing plant effluents. Anal. Chem. 49: 1433.

Gatz, E.E., and J.R. Jones. 1970. Haloperidal antagonism to the hyperpyrexic and lethal effects of 2,4-dinitrophenol in rats. Anesthesia Analgesia 49: 773.

Gehring, P.J., and J.F. Buerge. 1969a. The cataractogenic activity of 2, 4-dinitrophenol in ducks and rabbits. Toxicol. Appl. Pharmacol. 14: 475.

Gehring, P.J., and J.F. Buerge. 1969b. The distribution of 2, 4-dinitrophenol relative to its cataractogenic activity in ducklings and rabbits. Toxicol. Appl. Pharmacol. 15: 574.

Geiger, J.C. 1933. A death from alpha-dinitrophenol poisoning. Jour. Am. Med. Assoc. 101: 1333.

Gibson, J.E. 1973. Teratology studies in mice with 2-sec-butyl-4, 6-dinitrophenol (dinoseb). Food Cosmet. Toxicol. 11: 31.

Gisclard, J.B., and M.M. Woodward. 1946. 2,4-dinitrophenol poisoning: A case report. Jour. Ind. Hyg. Toxicol. 28: 47.

Gleason, M.N., et al. 1968. Clinical toxicology of some commercial products. Williams and Wilkins Co., Baltimore.

Gleason, M.N., et al. 1969. Clinical toxicology of commercial products. 3rd. ed. Williams and Wilkins Co., MacMillan Co.

Gomaa, H.M., and S.D. Faust. 1972. Chemical hydrolysis and oxidation of parathion and paraoxon in aquatic environments. Adv. Chem. Ser. Vol. III, Iss. Fate of Org. Pestic. in the Aquatic Environ. p. 189.

Grant, C.M. 1959. The action of nitrophenols on the pulmonary ventilation of rats. Br. Jour. Pharmacol. 14: 401.

Gunderson, K., and H.L. Jensen. 1956. A soil bacterium decomposing organic nitro-compounds. Acta Agric. Scand. 6: 100.

Haller, H.D. 1978. Degradation of mono-substituted benzoates and phenols by wastewater. Jour. Water Pollut. Control Fed. 50: 2771.

Harris, A.H., et al. 1946. Hematuria due to picric acid poisoning at a naval anchorage in Japan. Am. Jour. Pub. Health 36: 727.

Harvey, D.G. 1952. The toxicity of the dinitro-creosols, Part I. 4,6-dinitro-ortho-creosol and its simpler derivatives. Jour. Pharm. Pharmacol. 4: 1062.

Harvey, D.G. 1959. On the metabolism of some aromatic nitro compounds by different species of animal. Part III. The toxicity of the dinitrophenols, with a note on the effects of high environmental temperatures. Jour. Pharm. Pharmacol. 11: 462.

Harvey, D.G., et al. 1951. Poisoning by dinitro-ortho-cresol. Some observations on the effects of dinitro-ortho-cresol administration by mouth to human volunteers. Br. Med. Jour. 2: 13.

Heenan, M.P., and J.N. Smith. 1974. Water-soluble metabolites of p-nitrophenol and 1-naphthyl n-methylcarbomate in flies and grass grubs. Biochem. Jour. 144: 303.

Hoch, F.L. 1965. Synergism between calorigenic effects: L-thyroxine and 2,4-dinitrophenol or sodium salicylate in erthyroid rats. Endocrinology 76: 335.

Hoecker, J.E., et al. 1977. Information profiles on potential occupational hazards. Natl. Inst. Occup. Safety Health, Cincinnati, Ohio.

Hoffsommer, J.C., and J.M. Rosen. 1973. Hydrolysis of explosives in sea water. Bull. Environ. Contam. Toxicol. 10: 78.

Horner, W.D. 1942. Dinitrophenol and its relation to formation of cataracts. Arch. Ophthal. 27: 1097.

Horner, W.D., et al. 1935. Jour. AMA 105: 108.

Howard, H., et al. 1976. Investigation of selected potential environmental contamination: Nitroaromatics. Off. Tox. Subst. U.S. Environ. Prot. Agency, Washington, D.C.

Huidobro, F. 1971. Action of picric acid on the effects of some drugs acting on the central nervous system, with special reference to opiods. Arch. Int. Pharmacodyn Ther. 192: 362.

Hunter, D. 1950. Devices for the protection of the worker against injury and disease. Br. Med. Jour. 1: 449.

Jenkins, S., and H. Hawkes. 1961. Developments in biological filtration in Great Britain. Air Water Pollut. 5: 407.

Joiner, R.L., and K.P. Baetcke. 1973. Comparative studies of thin layer chromatographic systems for the separation and identification of photoalteration products of parathion. Jour. AOAC 56: 338.

Katan, J., and E.P. Lichtenstein. 1977. Mechanisms of production of soil-bound residues of [^{14}C] parathion by microorganisms. Jour. Agric. Food Chem. 25: 1404.

King, E., and D.G. Harvey. 1953a. Some observations on the absorption and excretion of 4,6-dinitro-o-cresol (DNOC). 1. Blood dinitro-o-cresol levels in the rat and rabbit following different methods of absorption. Biochem. Jour. 53: 185

King, E., and D.G. Harvey. 1953b. Some observations on the absorption and excretion of 4,6-dinitro-o-cresol (DNOC). 2. The elimination of 4,6-dinitro-o-cresol by man and by animals. Biochem. Jour. 53: 196.

Knowles, M.E., et al. 1975. Phenols in smoked, cured meats: Nitrosation of phenols in liquid smokes and in smoked bacon. Jour. Sci. Food Agric. 26: 267.

Kouris, C.S., and J. Northcott. 1963. Aniline and its derivatives. Page 411 in Kirk-Othmer Encyclopedia of Chemical Technology Vol. 2. 2nd ed.

Kutz, F.W., et al. 1978. Survey of pesticide residues and their metabolites in urine from the general population. in K.R. Rao, ed. Pentachlorophenol: Chemistry, pharmacology and environmental toxicology. Plenum Press. New York.

Landauer, W., and E. Clark. 1964. Uncouplers of oxidative phosphorylation and teratogenic activity of insulin. Nature 204: 285.

Landsteiner, M.D., and A.A. DiSomma. 1940. Studies on the sensitization of animals with simple chemical compounds. VIII. Sensitization to picric acid; subsidiary agents and mode of sensitization. Jour. Exp. Med. 72: 361.

Langerspetz, K., and H. Tarkkonen. 1961. Metabolic and antithyroid effects of prolonged administration of dinitrophenol in mice. Ann. Med. Exp. Biol. Finniae 39: 287.

Lao, R.C., et al. 1973. Assessment of environmental problems using the combination of gas chromatography and quadrapole mass spectrometry. Sci. Total Environ. 2: 223.

Lawford, D.J., et al. 1954. On the metabolism of some aromatic nitro-compounds by different species of animals. Jour. Pharm. Pharmacol. 6: 619.

Levin, A., and J.H. Tjio. 1948. Induction of chromosome fragmentation by phenols. Hereditas 34: 453.

Levine, S. 1977. Role of tissue hypermetabolism in stimulation of ventilation by dinitrophenol. Jour. Appl. Physiol. 43: 72.

Litterst, C.L., et al. 1975. Comparison of in vitro drug metabolism by lung, liver and kidney of several common laboratory species. Drug Metabol. Dispos. 3: 259.

MacBryde, C.M., and B.L. Taussig. 1935. Functional changes in liver, heart and muscles and loss of dextrose tolerance resulting from didnitrophenol. Jour. AMA. 105: 13.

Machleidt, H., et al. 1972. The hydrophobic expansion of erythrocyte membranes by the phenol anesthetics. Biochim. Biophys. Acta 255: 178.

Maguire, H.C. 1973. The bioassay of contact allergens in the guinea pig. Jour. Soc. Cosmet. Chem. 24: 151.

Maguire, H.C., and M.W. Chase. 1972. Studies on the sensitization of animals with simple chemical compounds. XIII. Sensitization of guinea pigs with picric acid. Jour. Exp. Med. 135: 357.

Makhinya, A.P. 1964. Effect of certain nitrophenols on the organoleptic qualities and the sanitary conditions of water basins. Vopr. Gigieny Naselen Mest. Kiev, Sb. 5: 43.

Makhinya, A.P. 1969. Comparative hygienic and sanitary- toxicological studies of nitrophenol isomers in relation to their normalization in reservoir waters. Prom. Zagryazneniya Vodoemov. 9: 84.

Matsuguma, H.J. 1967. Nitrophenols. Page 888 in Kirk-Othmer Encylopedia Chemical Technology Vol. 13. 2nd ed.

McCann, J., et al. 1975. Detection of carcinogens as mutagens in the salmonella/microsome test. Proc. Natl. Acad. Sci. 72: 5133.

Metcalf, R.L. 1955. Organic insecticides, their chemistry and mode of action. Interscience Publishers, New York.

Mitra, A.B., and G.K. Manna. 1971. Effect of some phenolic compounds on chromosomes of bone marrow cells of mice. Indian Jour. Med. Res. 59: 1442.

Miyamoto, K., et al. 1975. Deficient myelination by 2, 4-dinitrophenol administration in early stage of development. Teratology 12: 204.

Moldeus, P., et al. 1976. Oxidative and conjugative metabolism of p-nitroanisole and p-nitrophenol in isolated rat liver cells. Acta Pharmacol. Toxicol. 39: 17.

Mosinska, K., and A. Kotarski. 1972. Determination of 2-isopropyl-4,6-dinitrophenol and 2,4-DNP in herbicides and in technical 2-isopropyl-4,6-DNP by TCL. Chemia Anal. 17: 327.

Motais, R., et al. 1978. Uncouplers of oxidative phosphorylation; a structure-activity study of their inhibitory effect on passive chloride permeability. Biochem. Biophys. Acta 510: 201.

Munnecke, D.M., and D.P.H. Hsieh. 1974. Microbial decontamination of parathion and p-nitrophenol in aqueous media. Appl. Microbiol. 29: 212.

Munnecke, D.M., and D.P.H. Hsieh. 1976. Pathways of microbial metabolism of parathion. Appl. Microbiol. 31: 63.

Myslak, Z., et al. 1971. Acute nitrobenzene poisoning: A case report with data on urinary excretion of p-nitrophenol and p-aminophenol. Arch. Toxicol. 28: 208.

Nagy, Z., et al. 1975. The correct mutagenic effect of pesticides on Escherichia Coli WP2 try-. Acta. Microbiol. Acad. Sci. Hung. 22: 309.

Nakagawa, M., and D.G. Crosby. 1974. Photodecomposition of nitrofen. Jour. Agric. Food Chem. 22: 849.

National Academy of Sciences. 1977. Drinking water and health. Washington, D.C.

National Institute for Occupational Safety and Health. 1978. Criteria for a recommended standard: Occupational exposure to dinitro-ortho-creosol. Dep. Health Edu. Welfare, Washington, D. C.

Newsweek. 1933. Diet and die with excess a-dinitrophenol. 2: 28.

Nojima, K., et al. 1975. Studies on photochemistry of aromatic hydrocarbons. Chemosphere 4: 77.

Nojima, K., et al. 1976. Studies on photochemistry of aromatic hydrocarbons III. Formation of nitrophenols by the photochemical reaction of toluene in the presence of nitrogen monoxide and nitrophenols in rain. Chemosphere 5: 25.

Ogino, S., and K. Yasukura. 1957. Biochemical studies of cataracts, VI. Production of cataracts in guinea pigs with dinitrophenol. Am. Jour. Opthal. 43: 936.

Parascandola, J. 1974. Dinitrophenol and bioenergetics: An historical perspective. Mol. Cell. Biochem. 5: 69.

Parker, V.H. 1952. Enzymic reduction of 2,4-dinitrophenol by rat-tissue homogenates. Biochem. Jour. 51: 363.

Parker, V.H., et al. 1951. Some observations on the toxic properties of 3,5-dinitro-ortho-creosol. Br. Jour. Ind. Med. 9: 226.

Patty, F.R. 1963. Industrial hygiene and toxicology. John Wiley and Sons, Inc., New York.

Perkins, R.G. 1919. A study of the munitions intoxications in France. Pub. Health Rep. 34: 2335.

Pitter, P. 1976. Determination of biological degradability of organic substances. Water Res. 10: 231.

Plotz, M. 1936. Dinitro-ortho-creosol: A metabolic stimulator and its toxic side-actions. N.Y. State Jour. Med. 36: 266.

Pollard, A.B., and J.F. Filbee. 1951. Recovery after poisoning with dinitro-ortho-creosol. Lancet 2: 618.

Pugh, P.M., and S.L. Stone. 1968. The effect of 2,4-dinitrophenol and related compounds on bile secretion. Jour. Physiol. 198: 39.

Pulkkinen, M.O. 1966a. Sulphate conjugation during development, in human, rat and guinea pig. Acta. Physiol. Scand. 66: 115.

Pulkkinen, M.O. 1966b. Sulphate conjugation during pregnancy and under the influences of cortisone. Acta. Physiol. Scand. 66: 120.

Quebbeman, A.J., and M.W. Anders. 1973. Renal tubular conjugation and excretion of phenol and p-nitrophenol in the chicken: Differing mechanisms of renal transfer. Jour. Pharmacol. Exp. Ther. 184: 695.

Ranklin, M.R. 1974. Simultaneous hepatic microsomal oxidation of p-nitroanisole and glucoronide conjugation of p-nitrophenol. Biochem. Soc. Trans. 2: 891.

Roan, C.C., et al. 1969. Blood cholinesterases, serum parathion concentrations and urine p-nitrophenol concentrations in exposed individuals. Bull. Environ. Contam. Toxicol. 4: 362.

Roberts, M.S., et al. 1977. Permeability of human epidermis to phenolic compounds. Jour. Pharm. Pharmacol. 29: 677.

Robinson, D., et al. 1951. Studies in detoxication 39. Nitro compounds (a) the metabolism of o-, m-, and p-nitrophenols in the rabbit. (b) the glucuronides of the mononitrophenols and observations on the anomalous optical rotations of triacetyl -o-nitrophenyl glucuronide and its methylester. Biochem. Jour. 50: 221.

Rogers, R.L. 1971. Absorption, translocation, and metabolism of p-nitrophenyltrifluoro-2-nitro-p-tolyl ether by soybeans. Jour. Agric. Food Chem. 19: 32.

Sanchez, E., and T.R.Tephly. 1974. Morphine metabolism. I. Evidence for separate enzymes in the glucoronidation of morphine and p-nitrophenol by rat hepatic microsomes. Drug Metab. Dispos. 2: 254.

Sax, N.I. 1968. Dangerous properties of industrial materials. 3rd ed. Van Nostrand Reinhold Co., New York.

Senszuk, W., et al. 1971. Excretion of 2,4-dinitrophenol and 2-amino-4-nitrophenol. Bromatol. Chem. Toksykol. 4: 453.

Sethunathan, N. 1973. Degradation of parathion in flooded acid soils. Jour. Agric. Food Chem. 21: 602.

Sethunathan, N., and T. Yoshida. 1973. A Flavobacterium sp. that degrades diazinon and parathion. Can. Jour. Microbiol. 19: 873.

Shafik, T., et al. 1973. Multiresidue procedure for halo- and nitrophenols. Measurement of exposure to biodegradable pesticides yielding these compounds as metabolites. Jour. Agric. Food Chem. 21: 295.

Shah, P.M. 1975. Biological treatment of sugary waste waters using the uncoupler 2,4-dinitrophenol. Ph.D. dissertation. Rutgers University.

Shah, P.M., et al. 1975. Enhancement of specific waste water treatment by the uncoupler 2,4-dinitrophenol. Jour. Food Sci. 40: 302.

Sharma, A.K., and S. Ghosh. 1965. Chemical basis of the action of creosols and nitrophenols on chromosomes. Nucleus 8: 183.

Sheetz, M.P., and S.J. Singer. 1976. Equilibrium and kinetic effects of drugs on the shapes of human erythrocytes. Jour. Cell. Biol. 70: 247.

Siddaramappa, R., et al. 1973. Degradation of parathion by bacteria isolated from flooded soil. Appl. Microbiol. 26: 846.

Sidwell, V.D., et al. 1974. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish, and mollusks. I. Protein, fat, moisture, ash, carbohydrate, energy value, and cholestrol. Mar. Fish. Rev. 36: 21.

Simon, E.W. 1953. Mechanisms of dinitrophenol toxicity. Biol. Rev. 28: 453.

Slater, E.C. 1962. Mechanisms of uncoupling of oxidative phosphorylation by nitrophenols. Comp. Biochem. Physiol. 4: 281.

Smith, J.N., et al. 1953. Studies in detoxication 48. Urinary metabolites of 4,6-dinitro-o-creosol in the rabbit. Biochem. Jour. 54: 225.

Smith, R.P., et al. 1967. Chemically induced methemoglobinemia in the mouse. Biochem. Pharmacol. 16: 317.

Sovljanski, M., et al. 1971. Intoxications with dinitro-ortho-creosol. Arh. Hig. Rada Toksikol. 22: 329.

Spector, W.S. 1956. Handbook of toxicology. W.B. Saunders, Co., Philadelphia.

Spencer, H.C., et al. 1948. Toxicological studies on laboratory animals of certain alkyl dinitrophenols used in agriculture. Jour. Ind. Hyg. Toxicol. 30: 10.

Springer, E.L., et al. 1977a. Chemical treatment of chips for outdoor storage. Evaluation of sodium n-methyldithiocarbomate and sodium 2,4-dinitrophenol treatment. Tapi 60: 88.

Springer, E.L., et al. 1977b. Evaluation of chemical treatments to prevent deterioration of wood chips during storage. Tapi 60: 93.

Steer, C. 1951. Death from dinitro-o-creosol. Lancet 1: 1419.

Stenback, F., and H. Garcia. 1975. Modifying effect of dimethyl sulfoxide and other chemicals on experimental skin tumor induction. Ann. N.Y. Acad. Sci. 243: 209.

Stockdale, M., and M.J. Selwyn. 1971. Influence of ring substitutes on the action of phenols on some dehydrogenases, phosphokinases and the soluble ATPase from mitochondria. Eur. Jour. Biochem. 21: 416.

Stofen, D. 1973. The maximum permissible concentrations in the U.S.S.R. for harmful substances in drinking water. Toxicology 1: 187.

Stott, H. 1956. Polyneuritis after exposure to dinitro-ortho-creosol. Br. Med. Jour. 1: 900.

Sudhakar-Barik, R., et al. 1976. Metabolism of nitrophenols by bacteria isolated from parathion amended flooded soil. Antonie van Leeuwenhock 42: 461.

Sulzberger, M.B., and F. Wise. 1933. Drug eruptions. II. Dermatitis eczematosa due to drugs. Arch. Dermatol. Syphilol. 28: 461.

Swamy, S.A. 1953. Suicidal poisoning by dinitrophenol. Jour. IMA, 22.

Swenberg, J.A., et al. 1976. In vitro DNA damage/akaline elution assay for predicting carcinogenic potential. Biochem. Biophys. Res. Commun. 72: 732.

Szybalski, W. 1958. Special microbiological systems. II. Observations on chemical mutagenesis in microorganisms. Ann. N.Y. Acad. Sci. 76: 475.

Tabak, H.H., et al. 1964. Microbial metabolism of aromatic compounds. I. Decomposition of phenolic compounds and aromatic hydrocarbons by phenol-adapted bacteria. Jour. Bacteriol. 87: 910.

Tainter, M.L., and W.C. Cutting. 1933. Miscellaneous actions of dinitrophenol. Repeated administration, antedates, fatal doses, antiseptic tests and actions of some isomers. Jour. Pharmacol. Exp. Ther. 49: 187.

Tainter, M.L., et al. 1933. Use of dinitrophenol in obesity and related conditions. Jour. Am. Med. Assoc. 101: 1472.

Tewfik, M.S., and Y.A. Hamdi. 1975. Metabolism of fluorodifen by soil microorganisms. Soil Biol. Biochem. 7: 79.

Trifunovic, V., et al. 1971. Waste water from the pesticide industry. Zastika Materijala 19: 381.

Truhaut, R., and E. De Lavaur. 1967. Study of 4, 6-dinitro-ortho-creosol metabolism in rabbits. C. R. Acad. Sci. (Paris) 264: 1937.

U.S. EPA. 1976. Frequency of organic compounds identified in water. U.S. Environ. Prot. Agency. Contract No. EPA 600/4-76-062.

U.S. International Trade Commission. 1967-73. Synthetic organic chemicals: U.S. production and sales. Washington, D.C.

U.S. International Trade Commission. 1976. Imports of benzenoid chemicals and products, 1974. Publ. No. 762. Washington, D. C.

Van Noort, et al. 1960. DNOC poisoning in Ag. sprayers. Nederlands Tijdscharift voor Geneeshunde 104: 676.

Veith, G.D., et al. An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish. (Manuscript.)

Vernot, E.H., et al. 1977. Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. Toxicol. Appl. Pharmacol. 42: 417.

Vestergaard-Bogind, B., and G. Lunn. 1977. Accelerating effect of 2,4,6-trinitrophenol on the glycolytic rate of human red cells. Jour. Gen. Physiol. 70: 661.

von Oettingen, W.P. 1949. Phenol and its derivatives. The relation between their chemical constitution and their effect on the organism. Natl. Inst. Health Bull. No. 190.

Weast, R.C., ed. 1975. Handbook of chemistry and physics. 57th ed. CRC Press.

Webb, R.G., et al. 1973. Current practices in GC-MS analysis of organics in water. EPA-R2-73-277. U.S. Environ. Prot. Agency, Washington, D. C.

Wedding, R.T., et al. 1967. Inhibition of malate dehydrogenase by phenols and the influence of ring substituents on their inhibitory effectiveness. Arch. Biochem. Biophys. 121: 9.

Williams, R.T. 1959. The metabolism and detoxication of drugs, toxic substances and other organic compounds. 2nd. ed. John Wiley and Sons, New York.

Windholz, M., ed. 1976. The Merck Index. 9th ed. Merck and Co., Rahway, N.J.

Wolfe, H.R., et al. 1970. Urinary excretion of insecticide metabolites: Excretion of paranitrophenol and DDA as indicators of exposure to parathion. Arch. Environ. Health 21: 711.

Wulff, L.M.B., et al. 1935. Some effects of alpha- dinitrophenol on pregnancy in the white rat. Proc. Soc. Exp. Biol. Med. 32: 678.

Yang, R.S.H., and C.F. Wilkinson. 1971. Conjugation of p-nitrophenol with sulfate in larvae of the southern army worm (Prodenia eridania). Pestic. Biochem. Physiol. 1: 327.

Yoshikawa, K., et al. 1976. Studies on the mutagenicity of hair-dye. Kokuritsu Eisei Shikenjo 94: 28.